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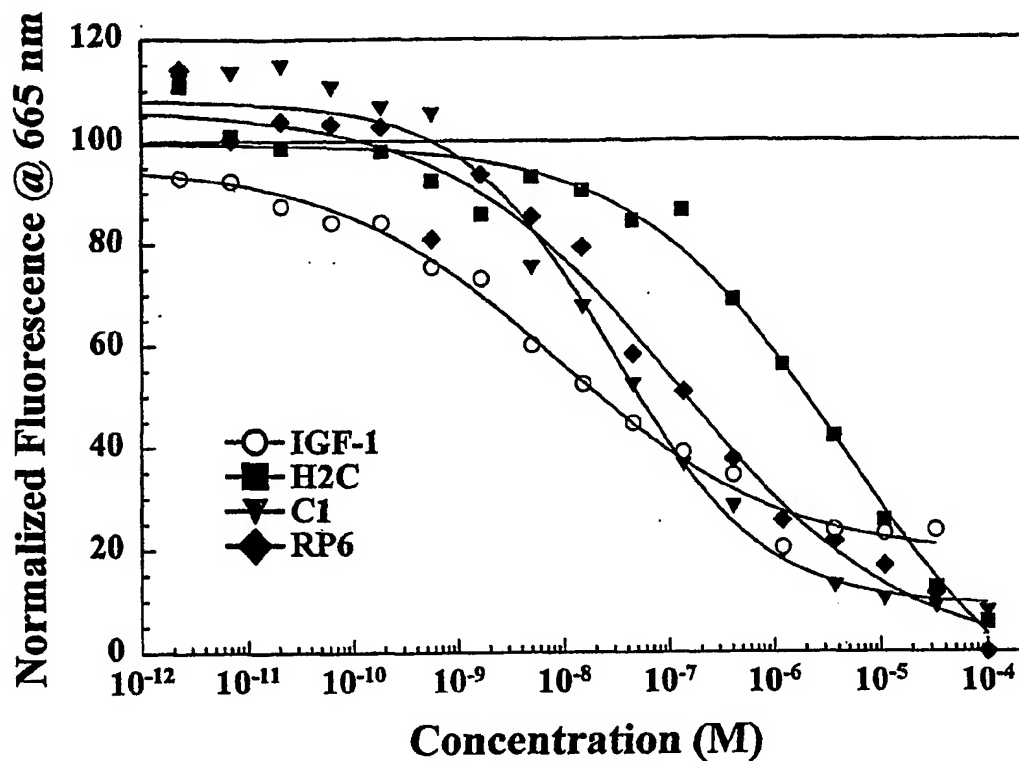


FIG. 73

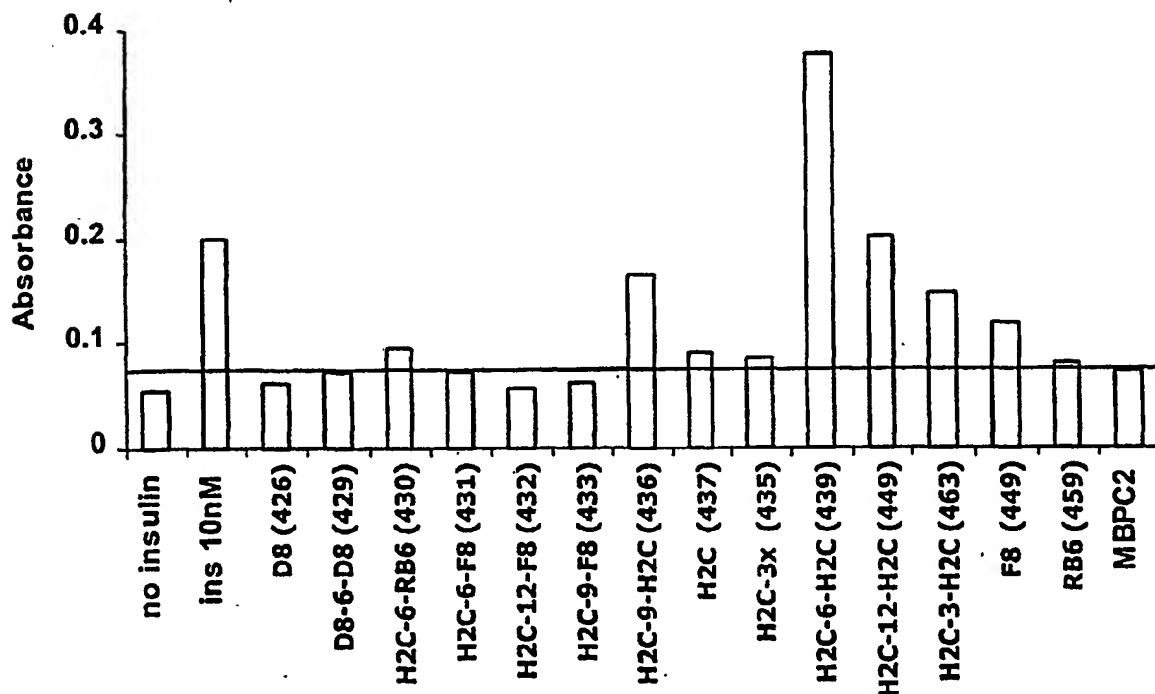


FIG. 74

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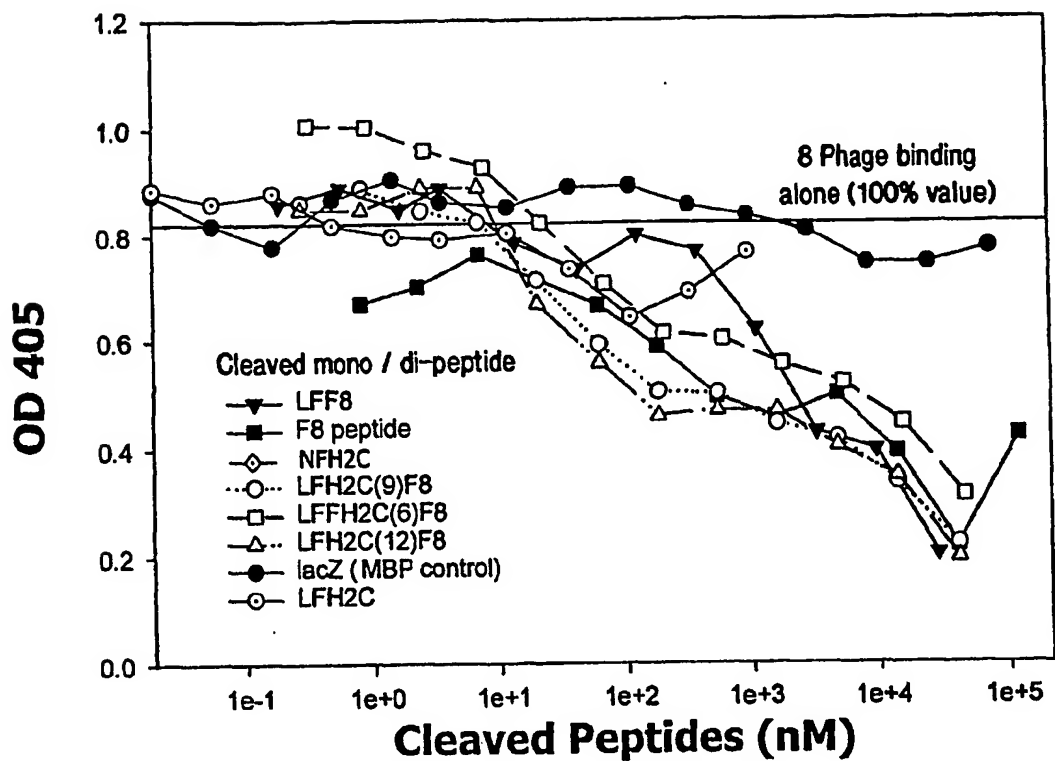


FIG. 72A

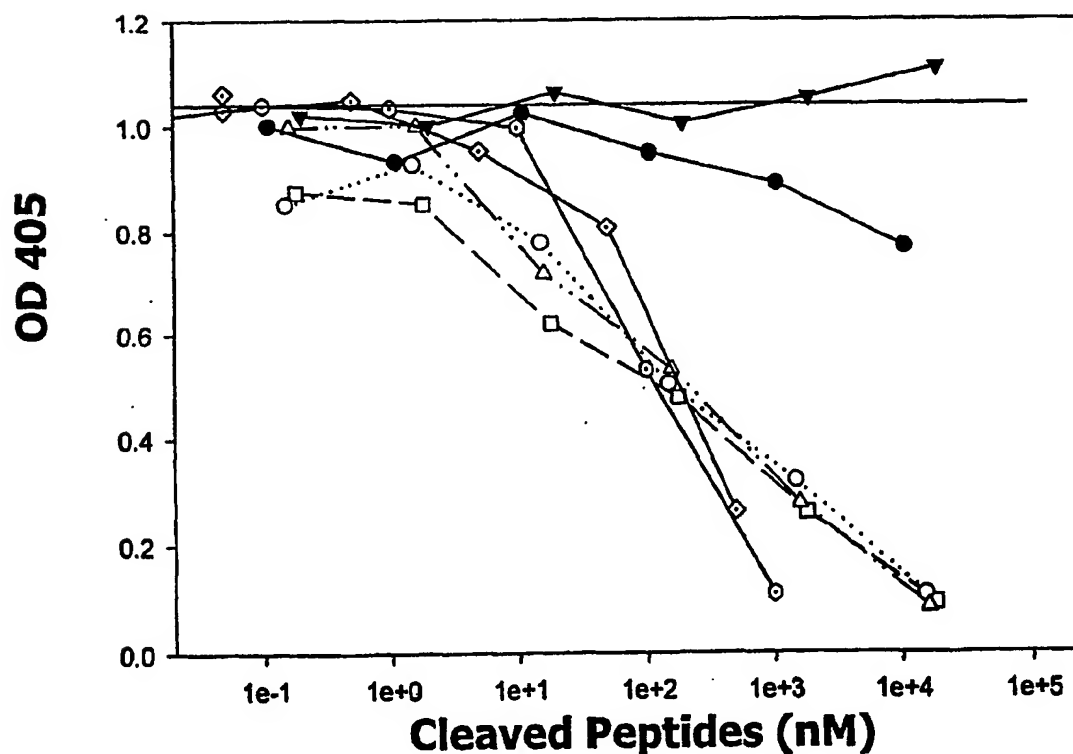


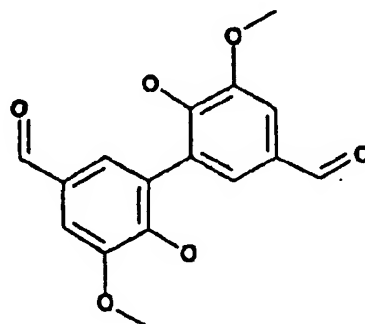
FIG. 72B

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S291: Dimer of S204 with linker 9

S204 = Lig-GGGFHENFYDWFVRQVSKK

Linker 9 =

HIR binding = $1.2 \cdot 10^{-6}$

FFC:

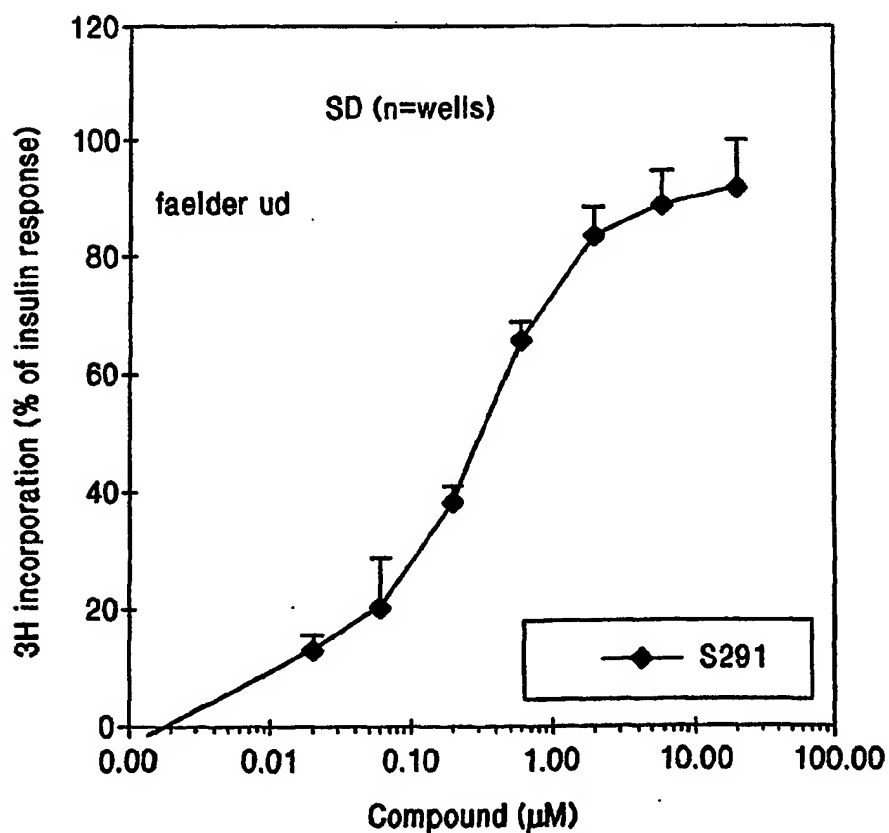


FIG. 71B3

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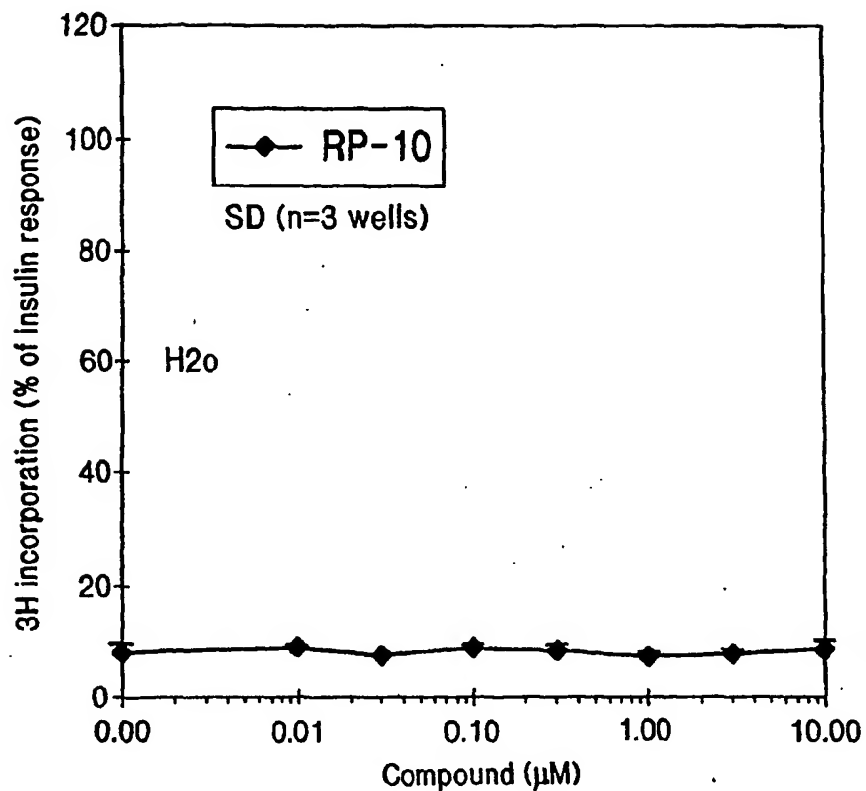


FIG. 71Z2

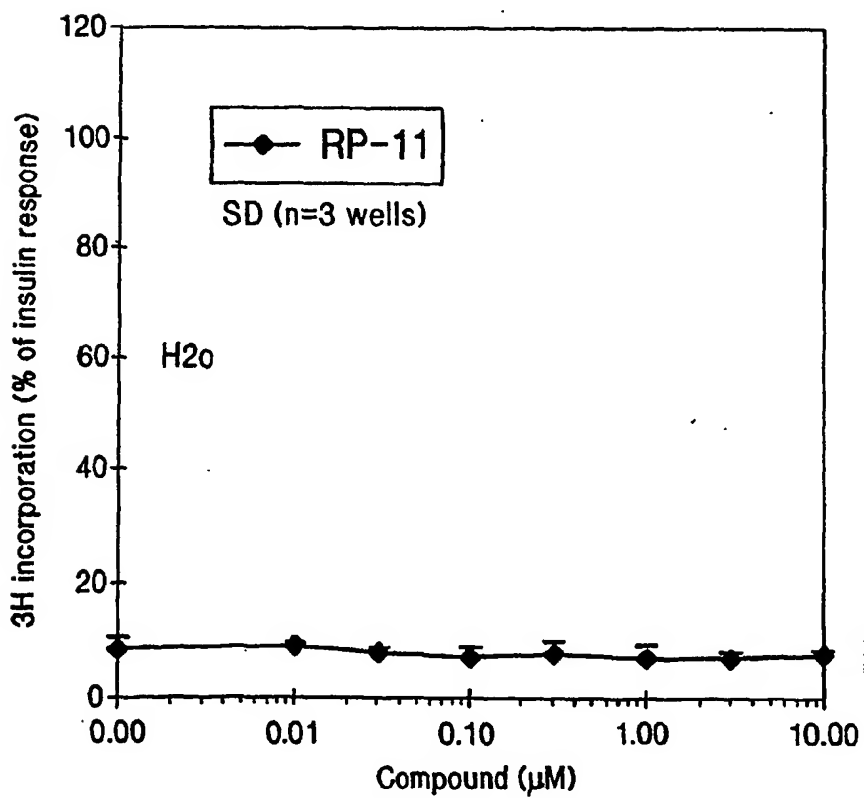


FIG. 71A3

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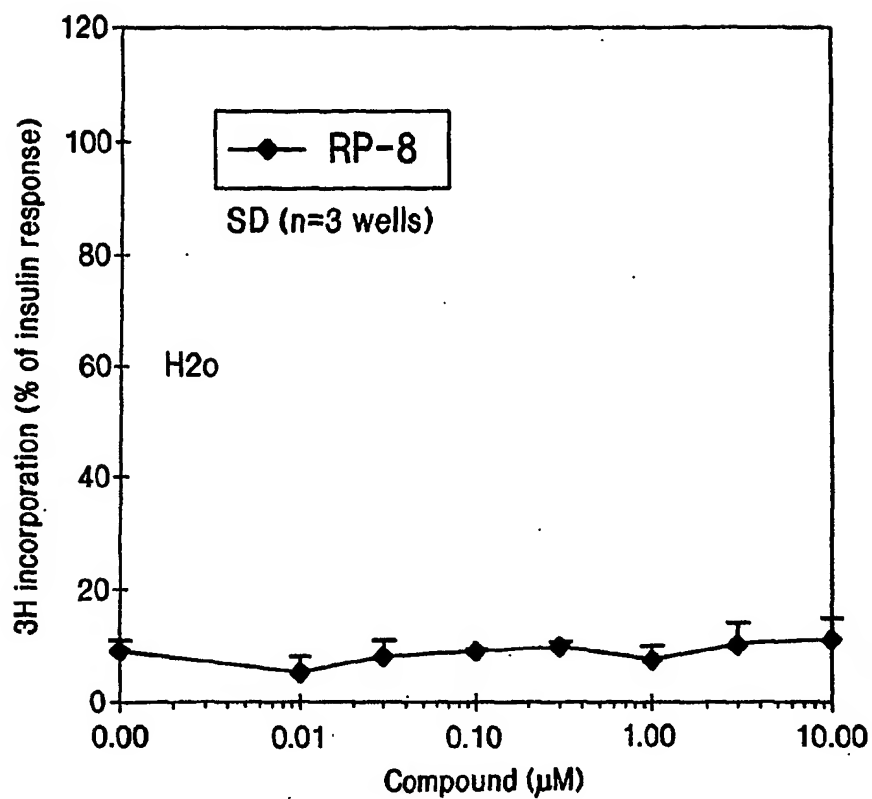


FIG. 71X2

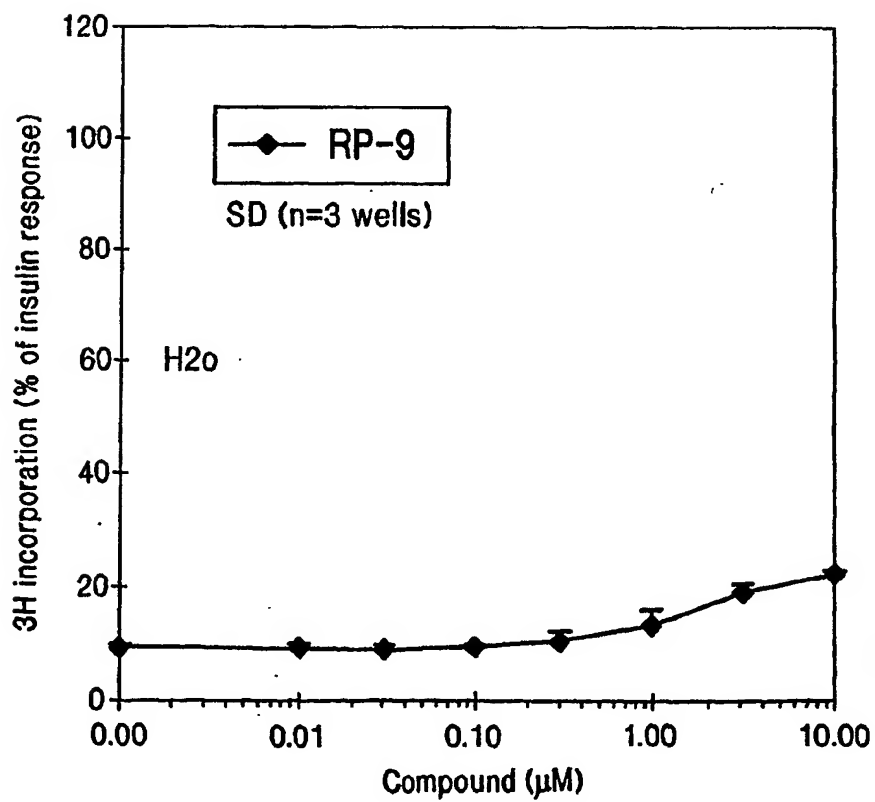


FIG. 71Y2

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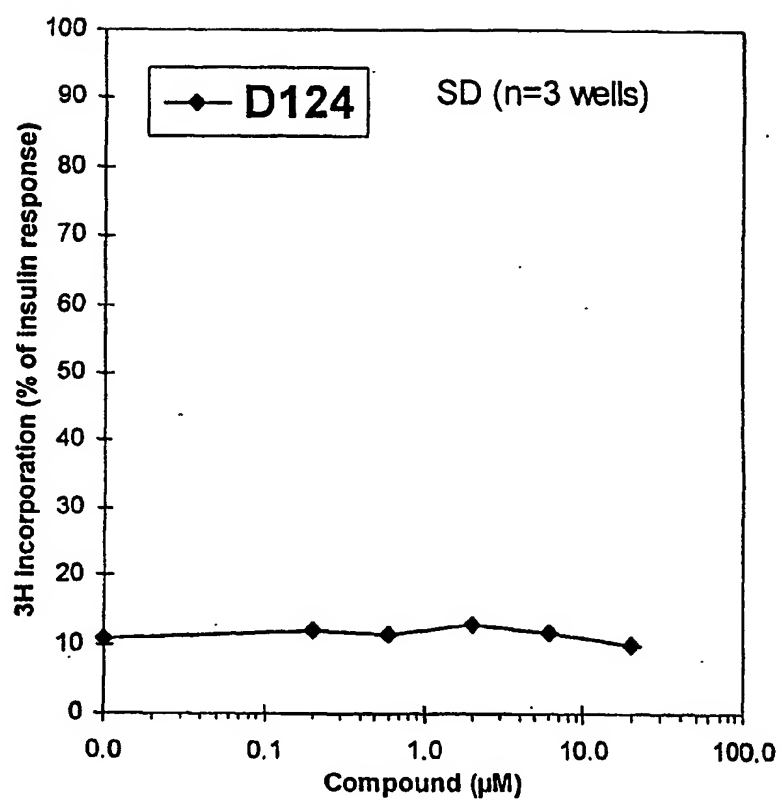


FIG. 71W2

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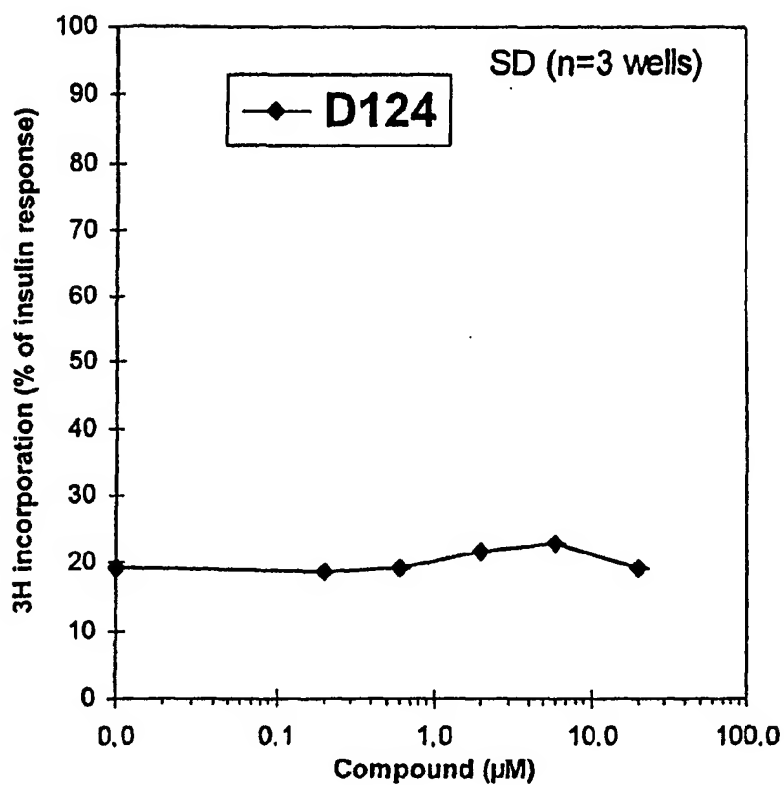


FIG. 71U2

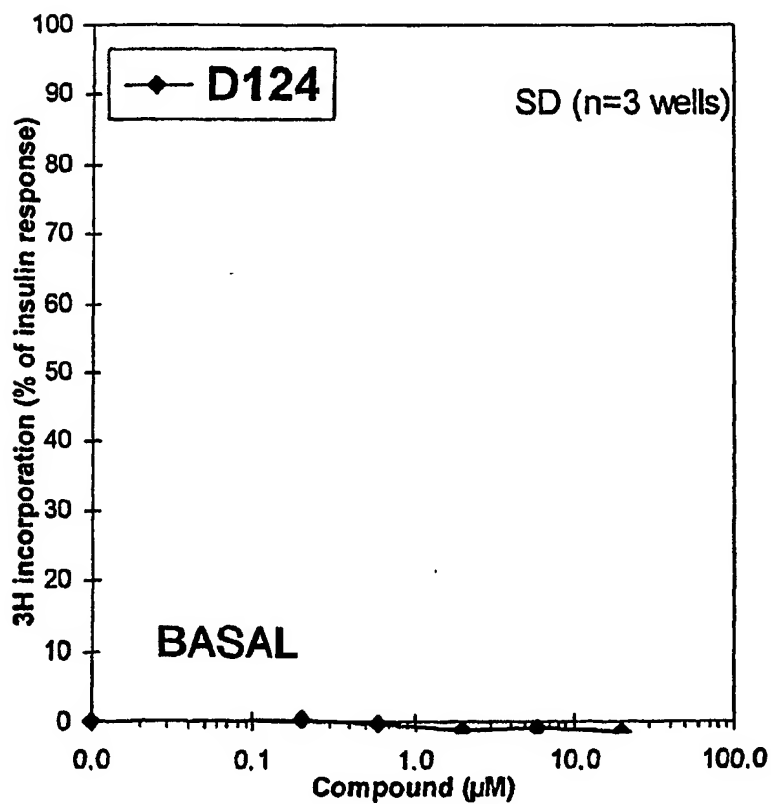


FIG. 71V2

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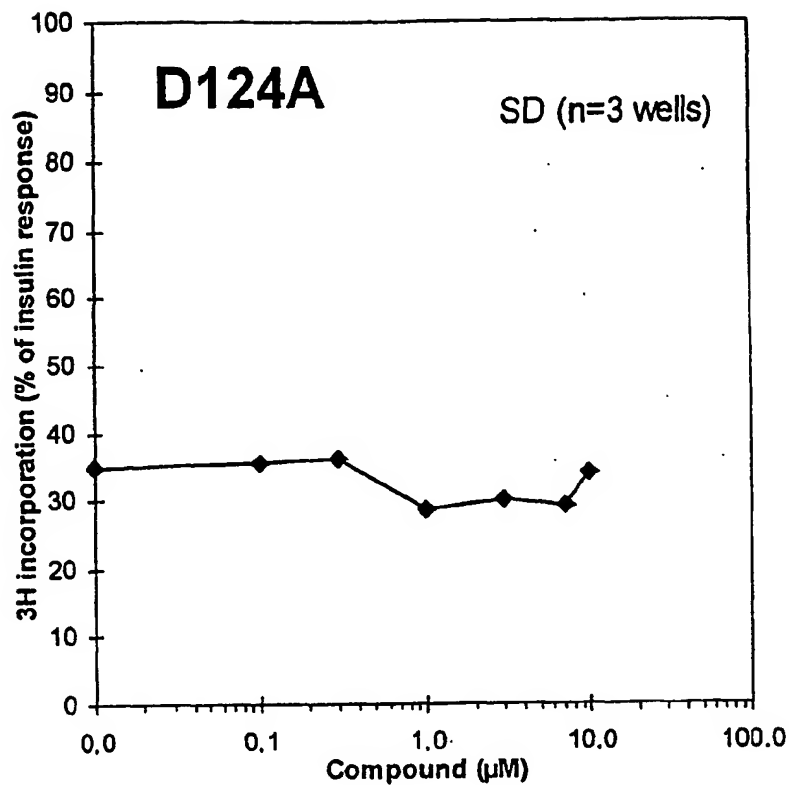


FIG. 71S2

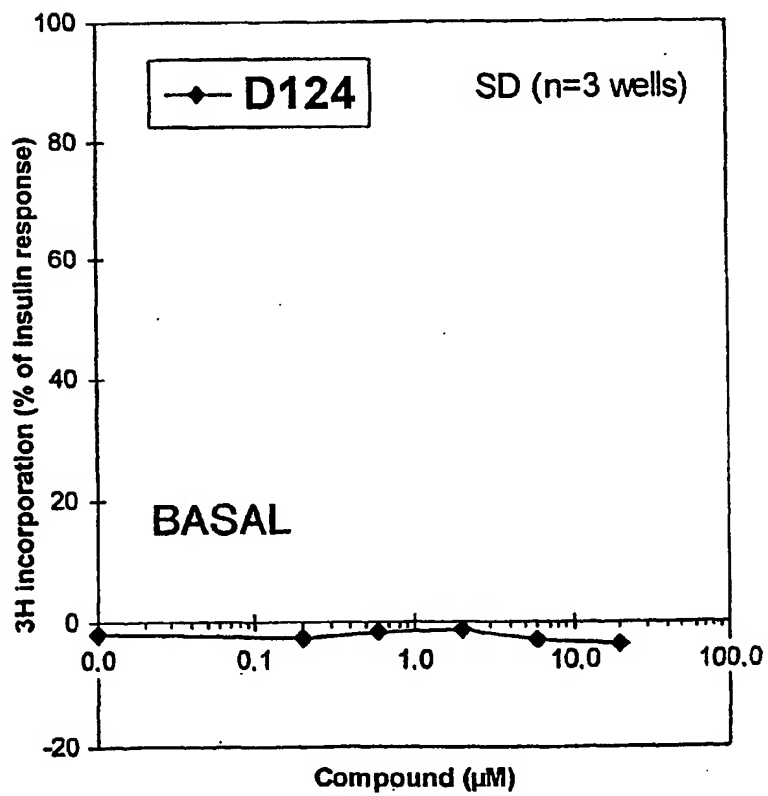


FIG. 71T2

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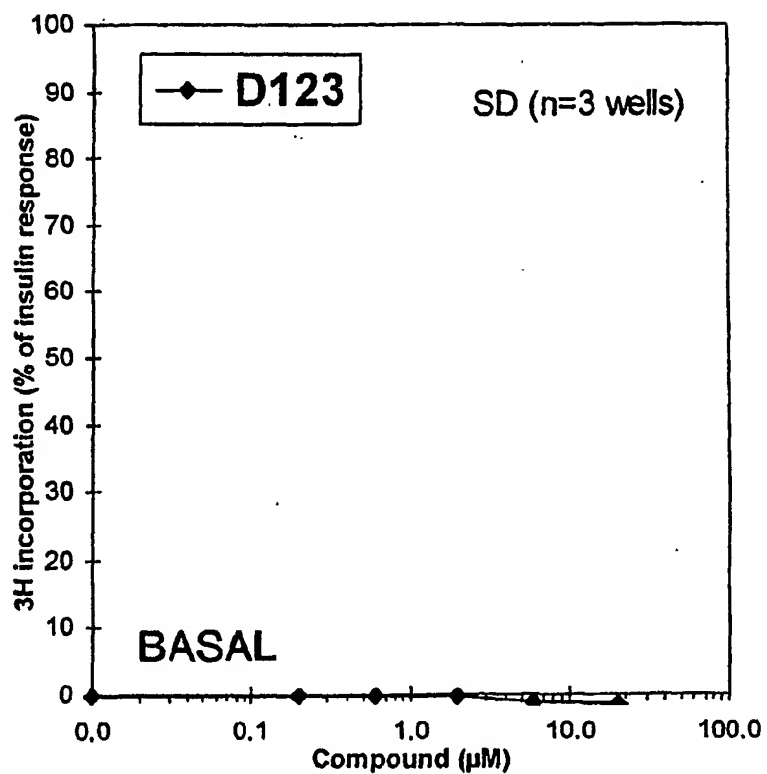


FIG. 71Q2

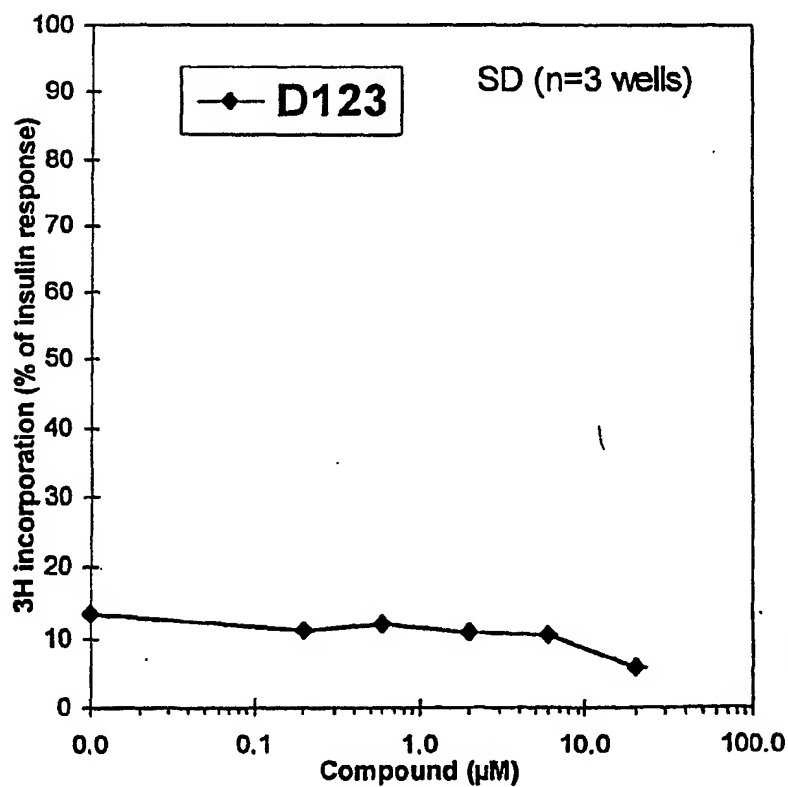


FIG. 71R2

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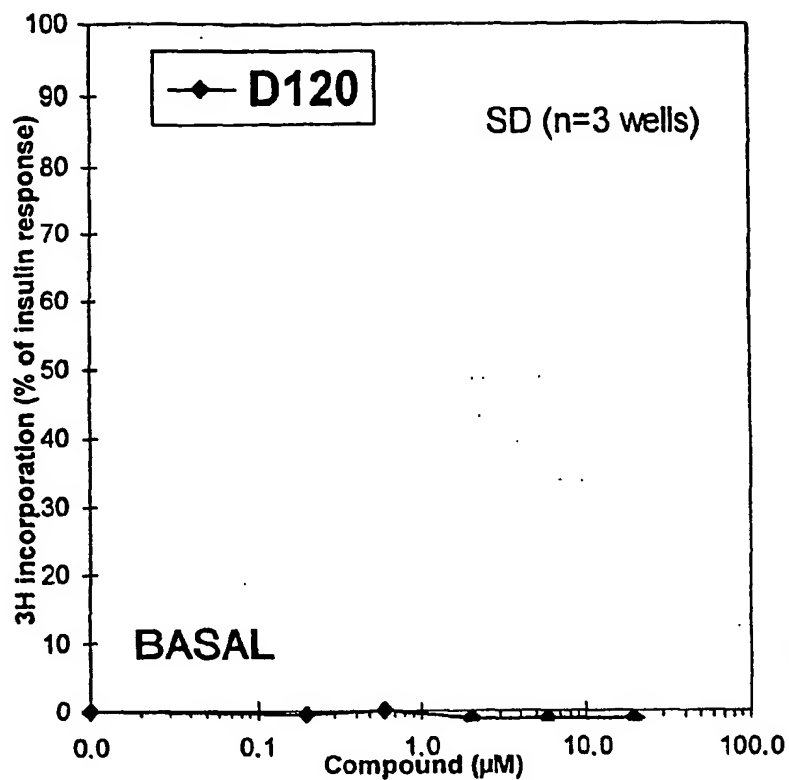


FIG. 71O2

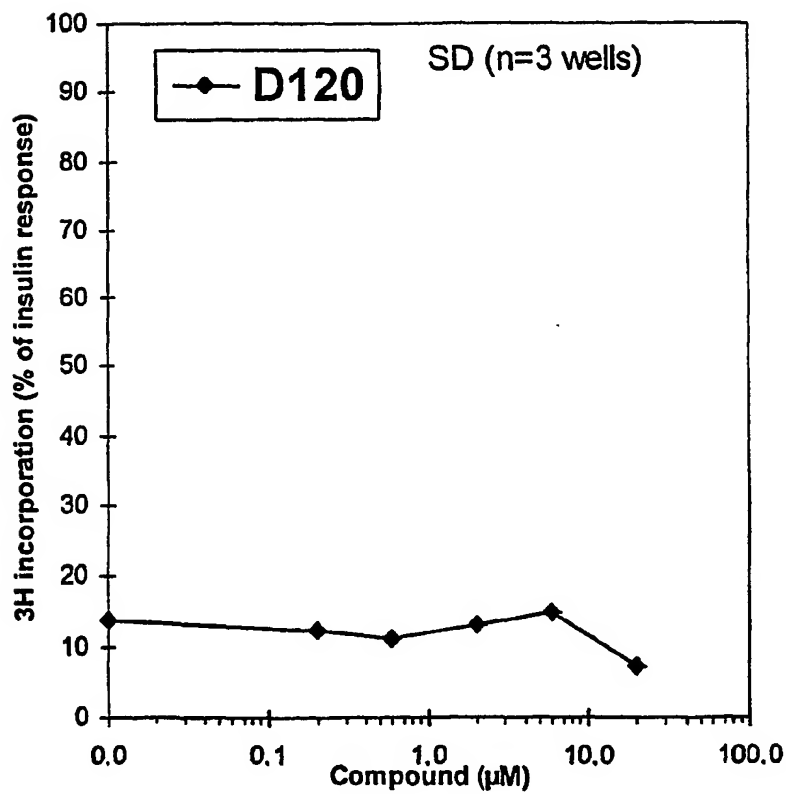


FIG. 71P2

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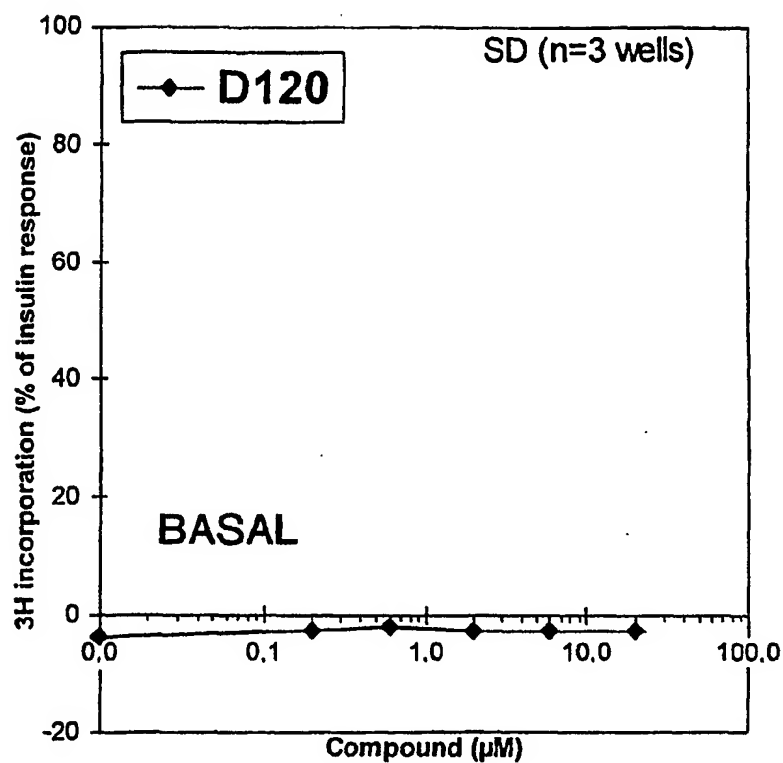


FIG. 71M2

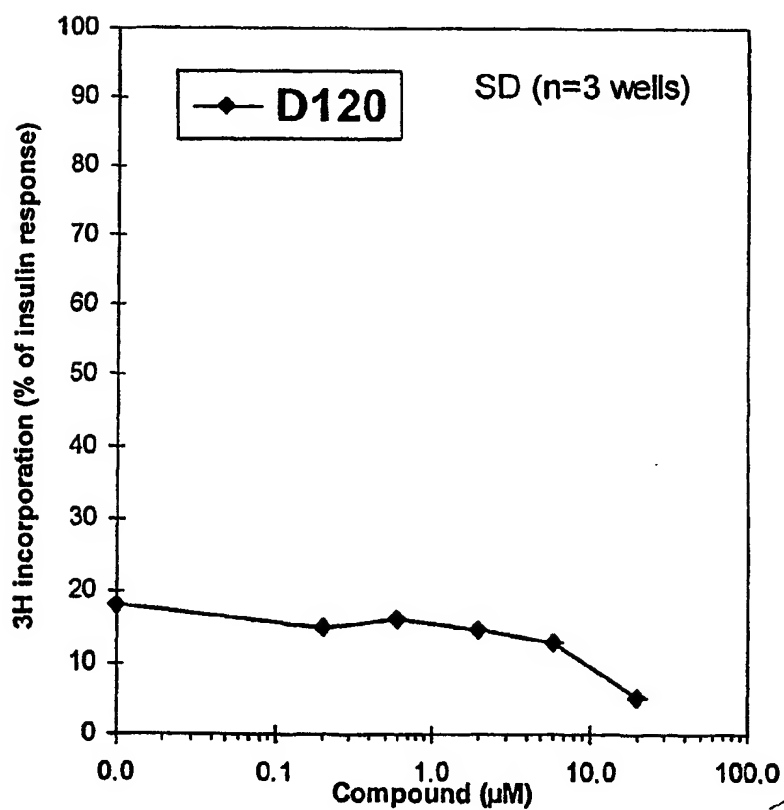


FIG. 71N2

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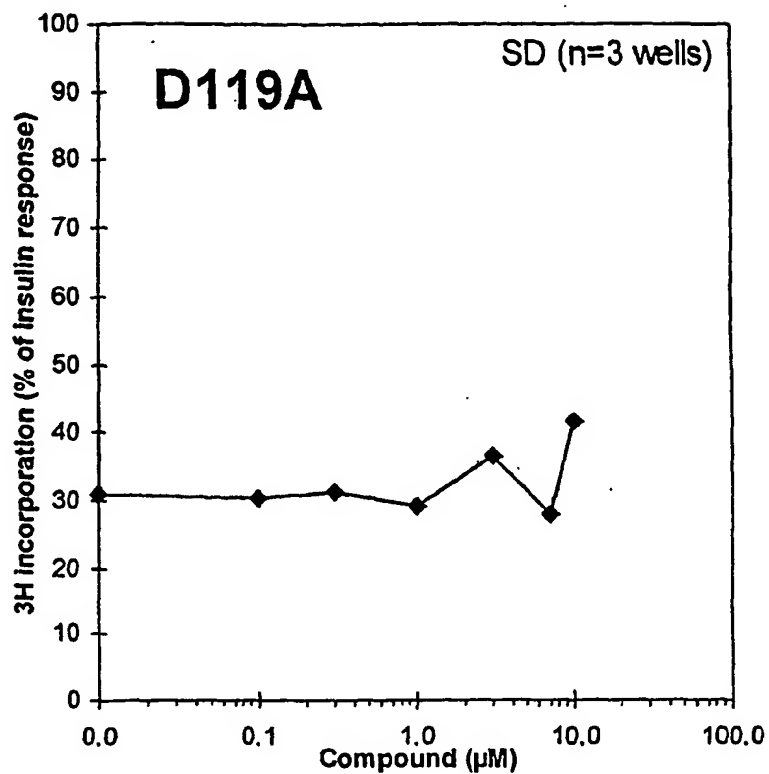


FIG. 71K2

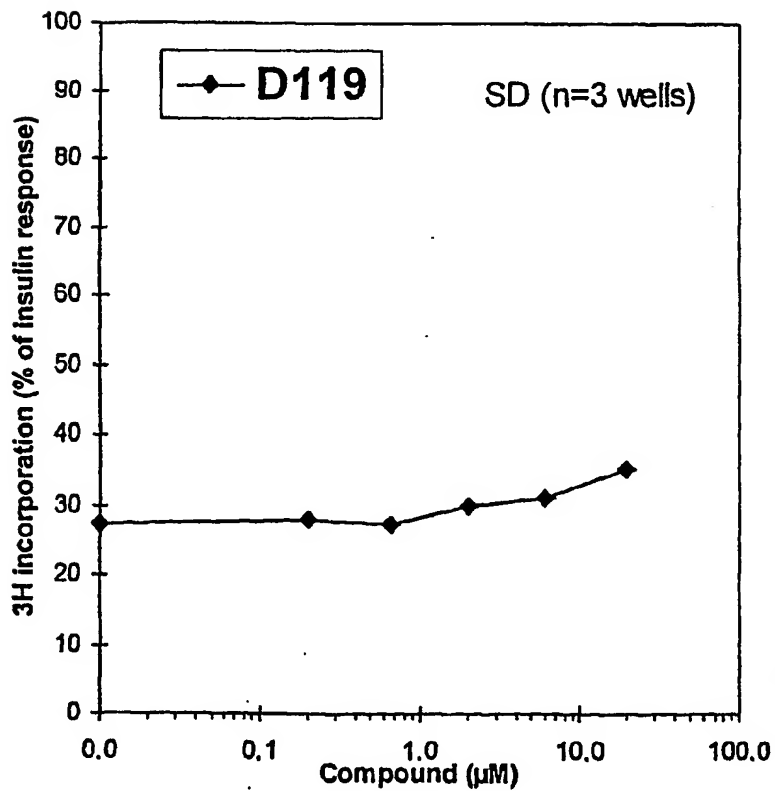


FIG. 71L2

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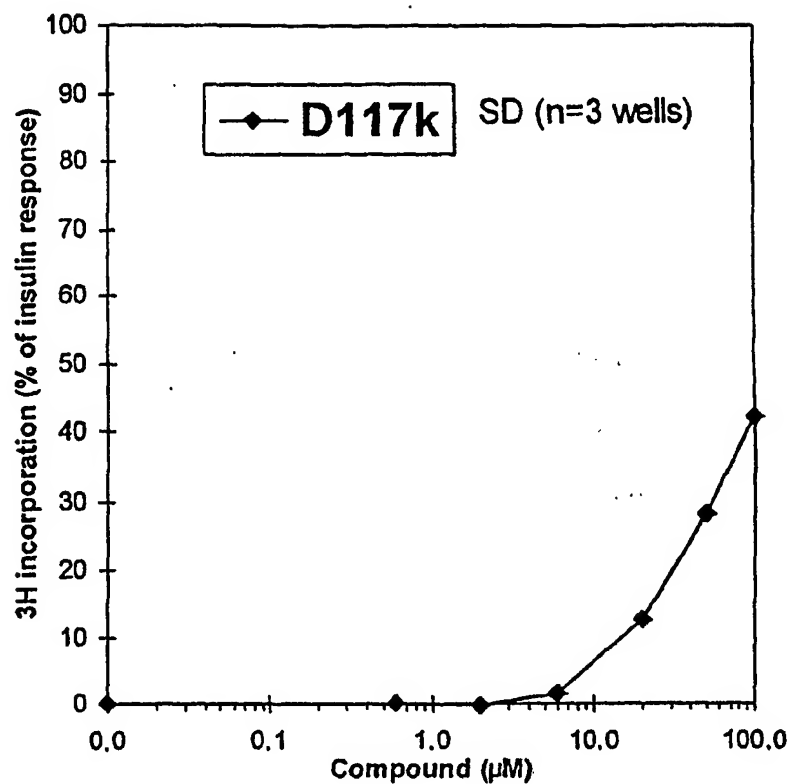


FIG. 71I2

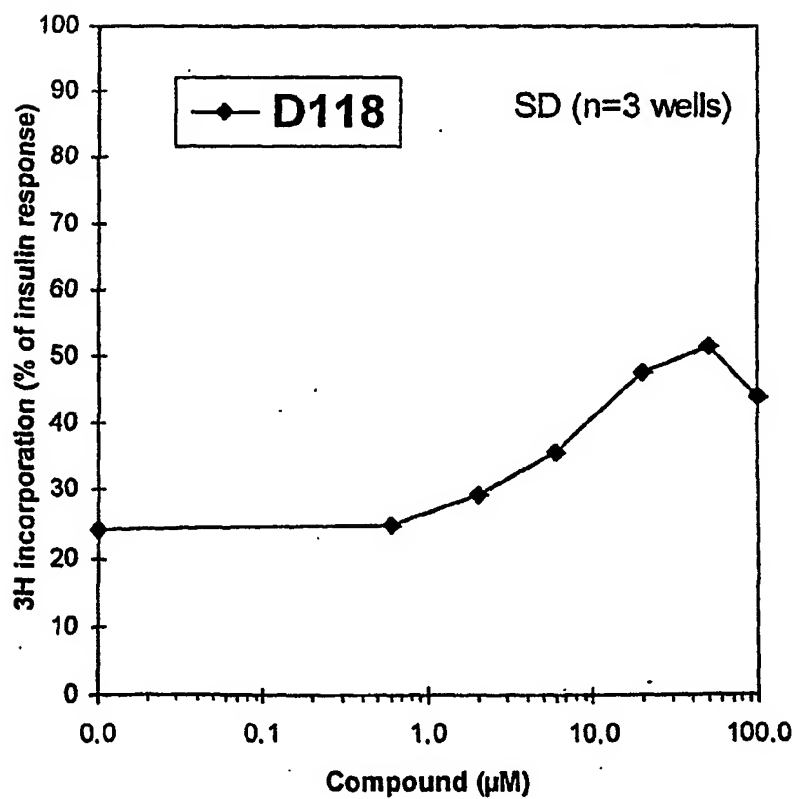


FIG. 71J2

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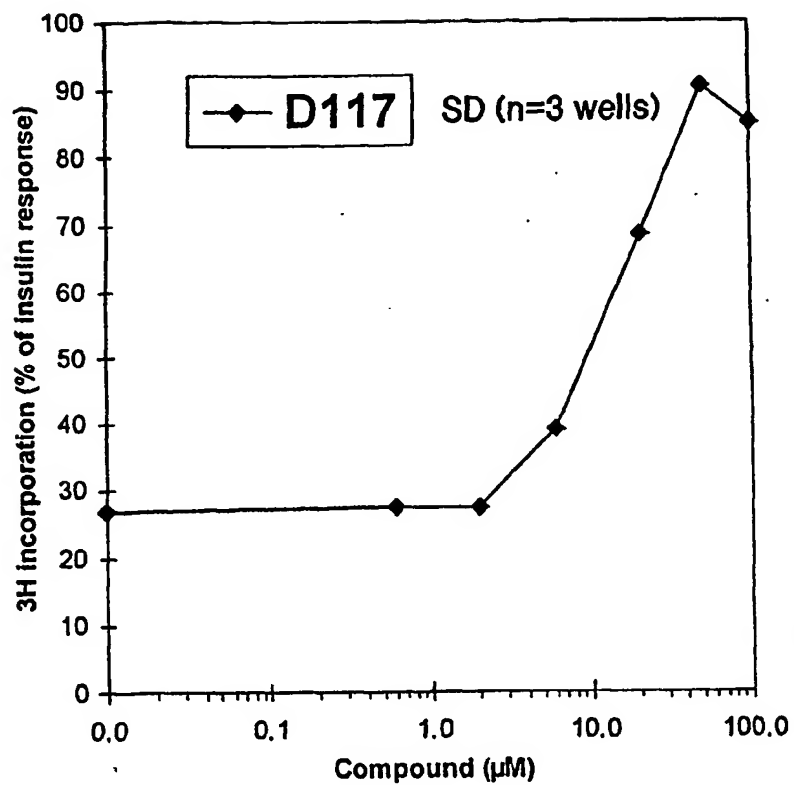


FIG. 71G2

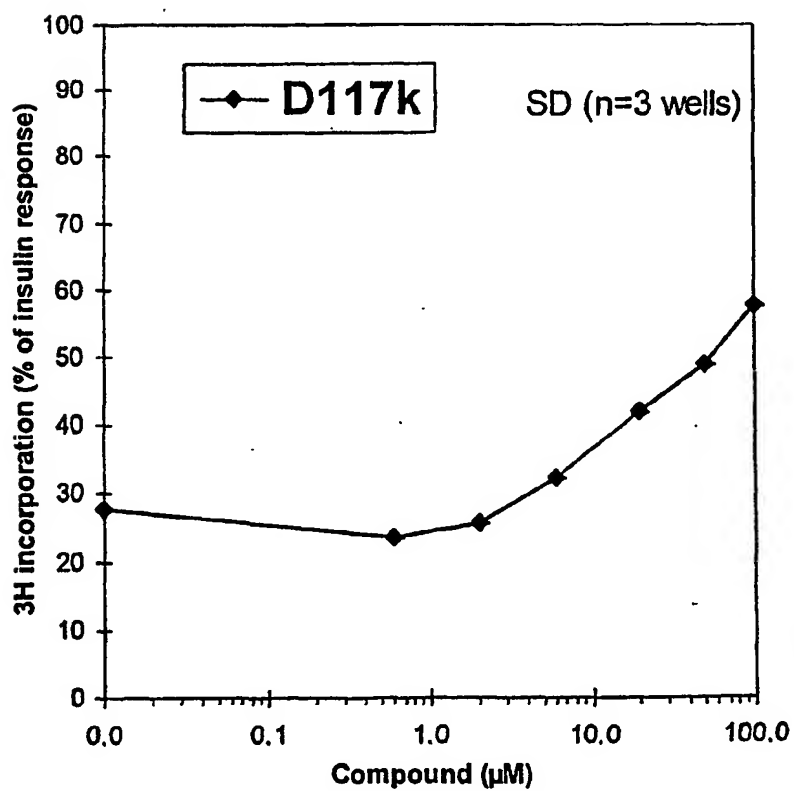


FIG. 71H2

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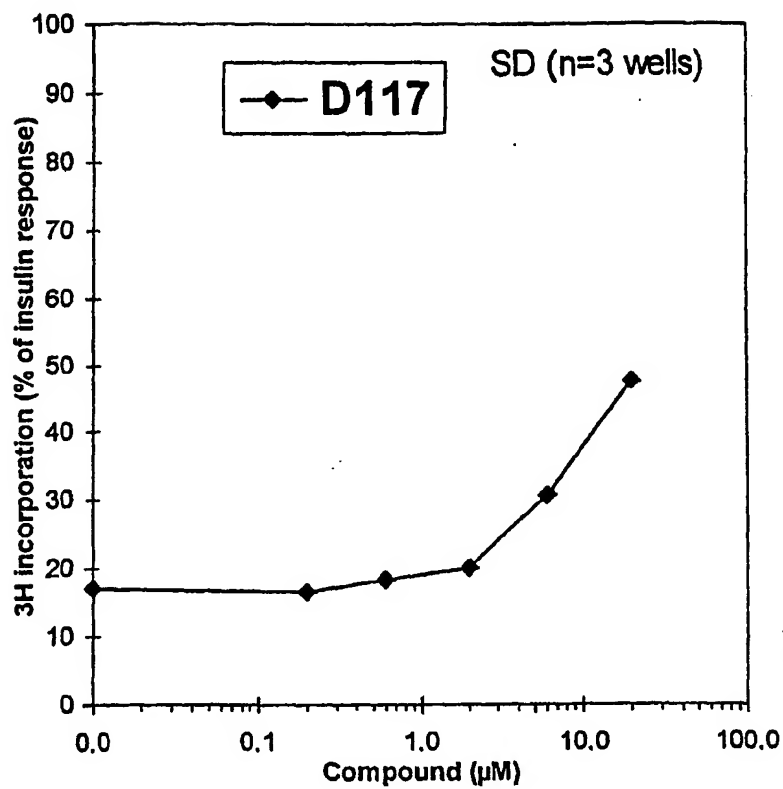


FIG. 71E2

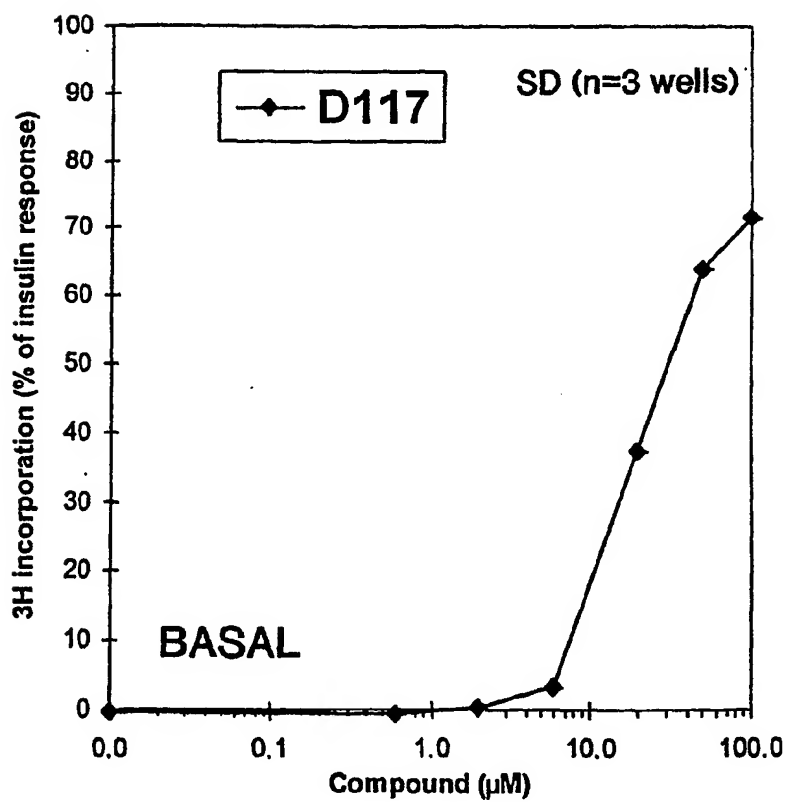


FIG. 71F2

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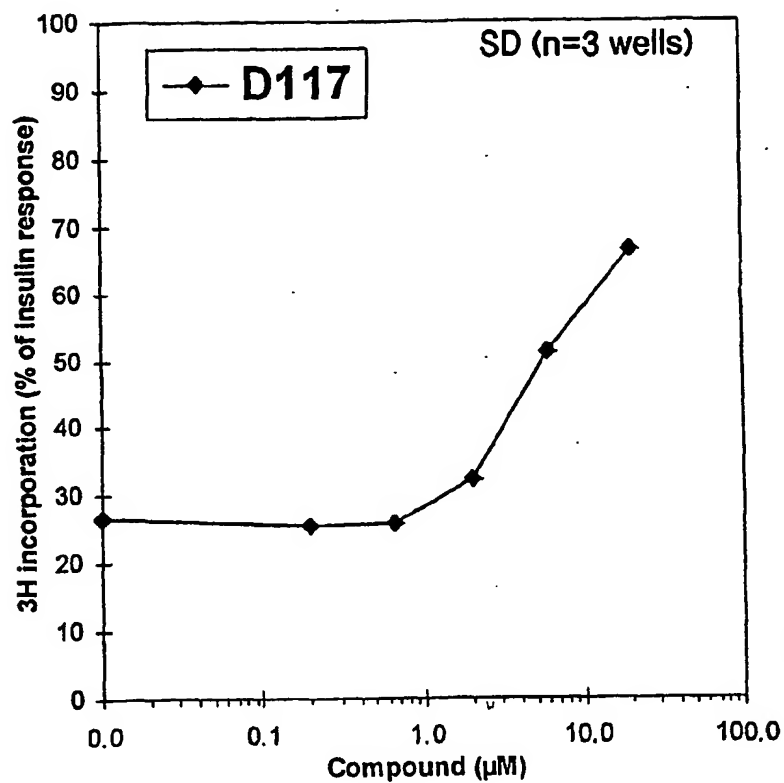


FIG. 71C2

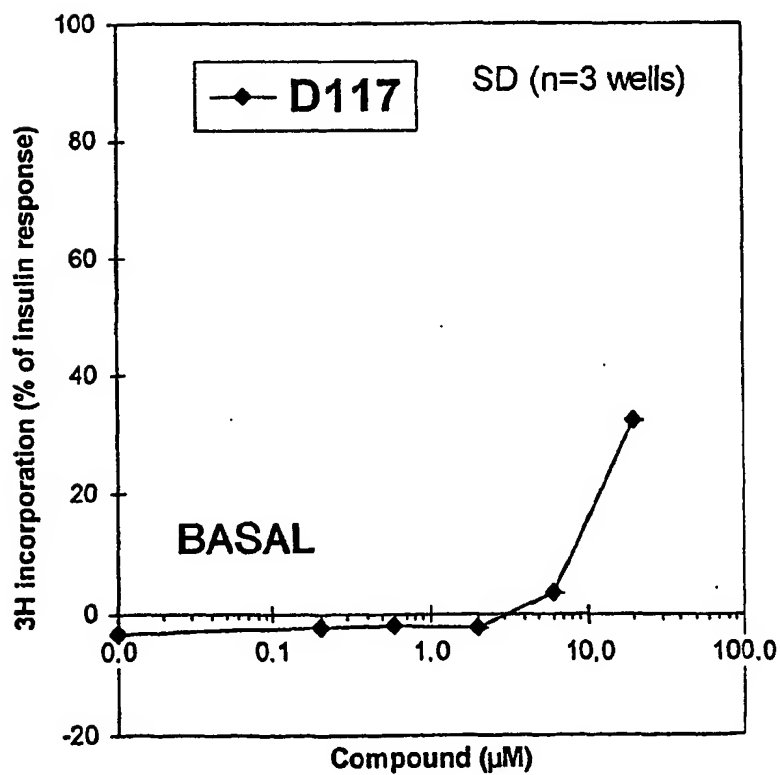


FIG. 71D2

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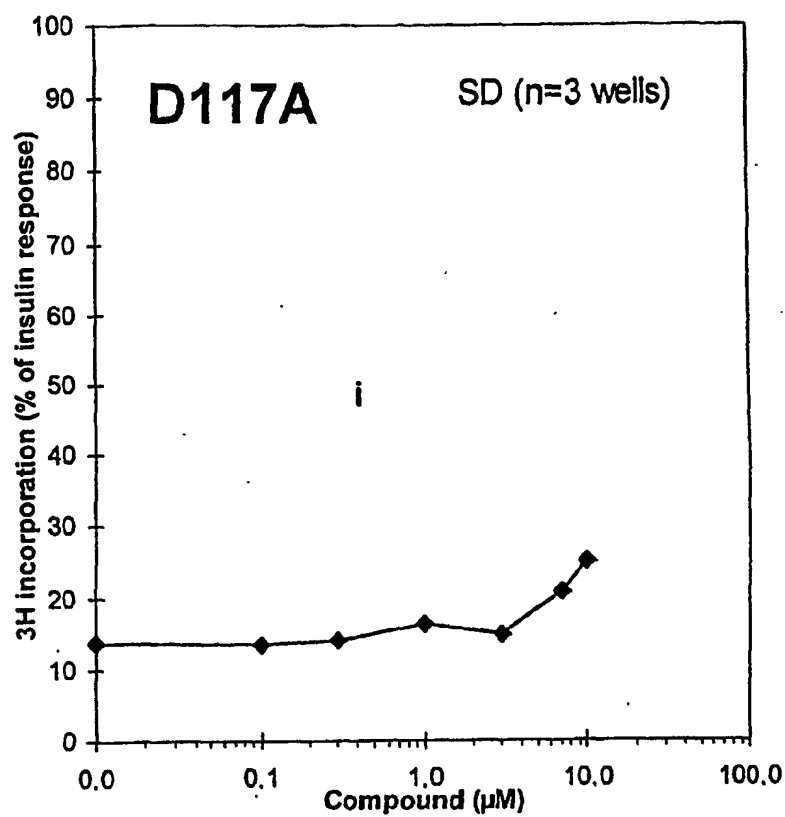


FIG. 71A2

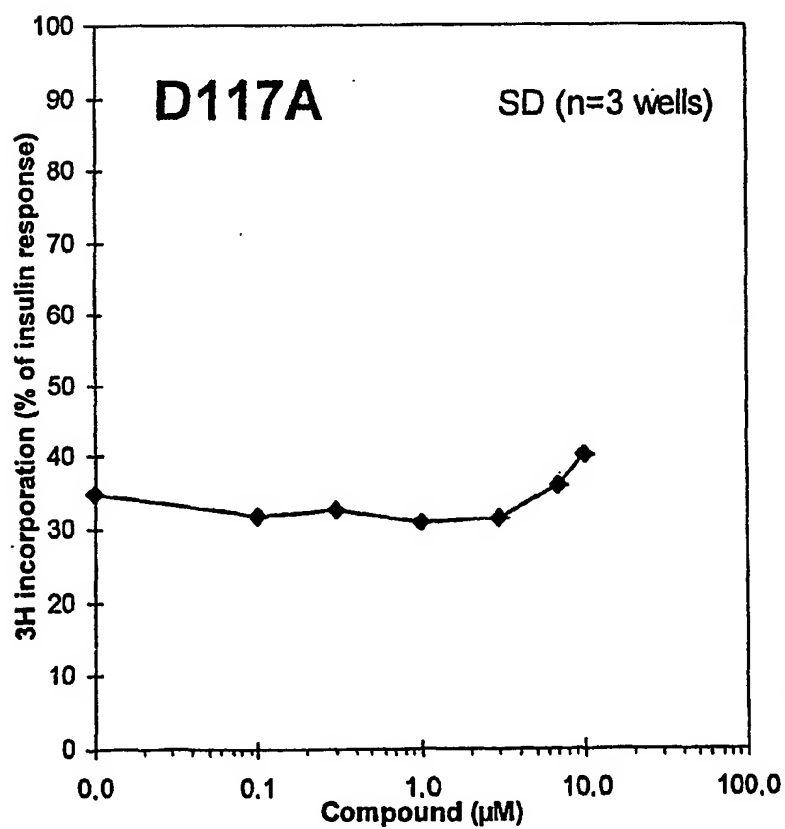


FIG. 71B2

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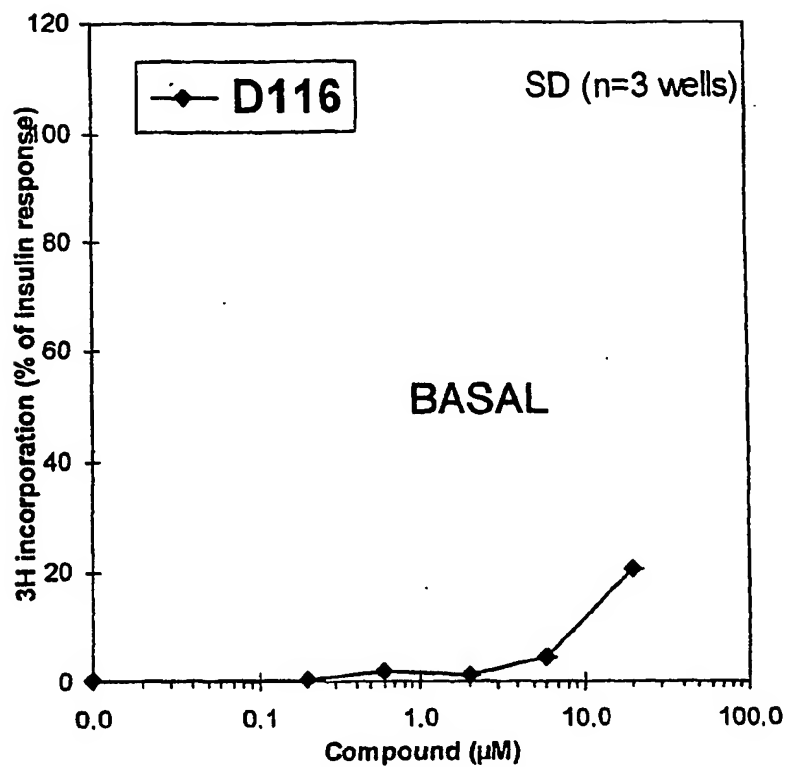


FIG. 71Y

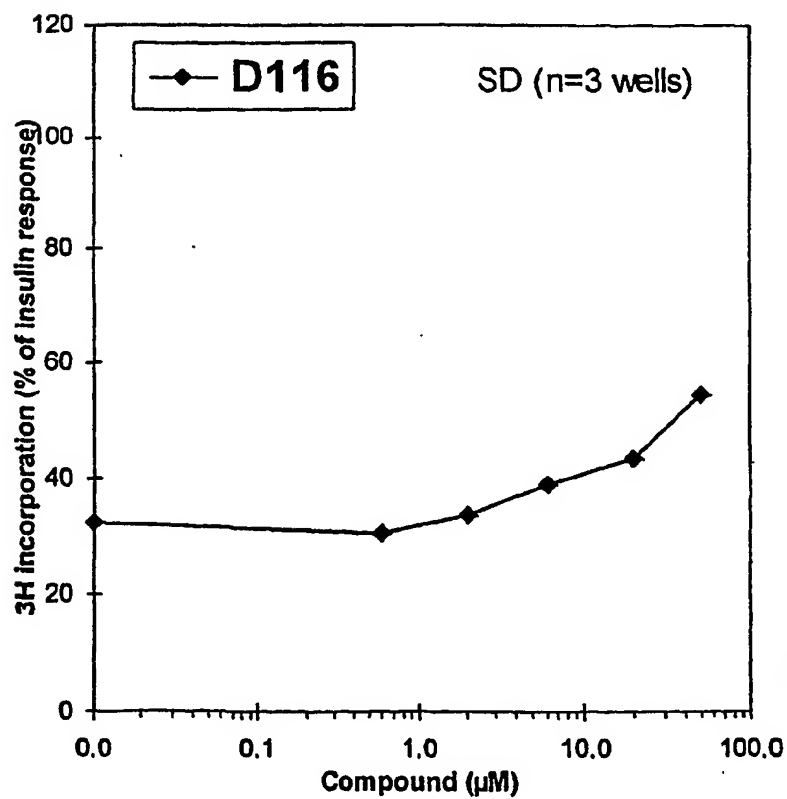


FIG. 71Z

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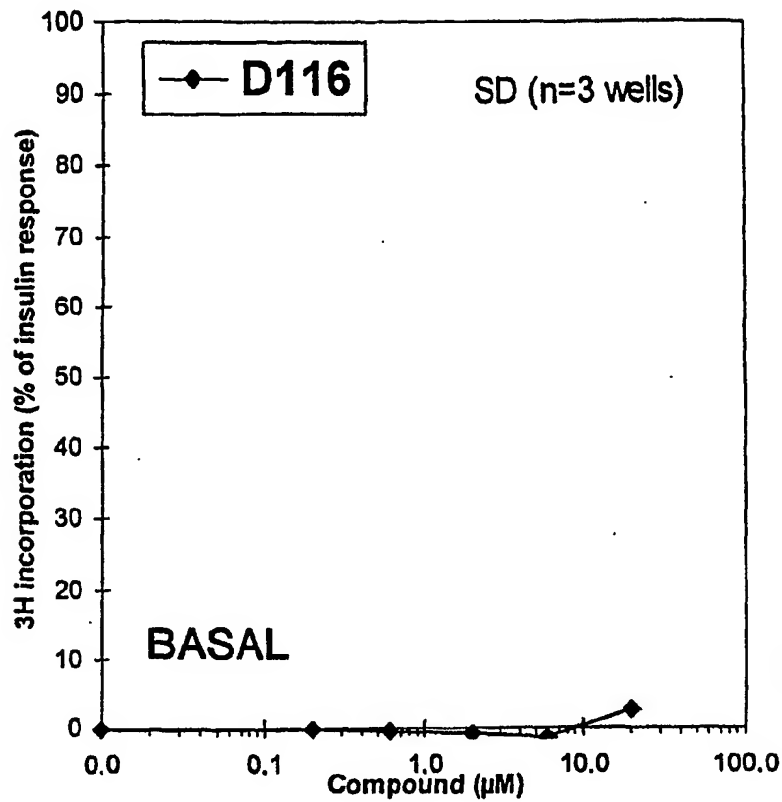


FIG. 71W

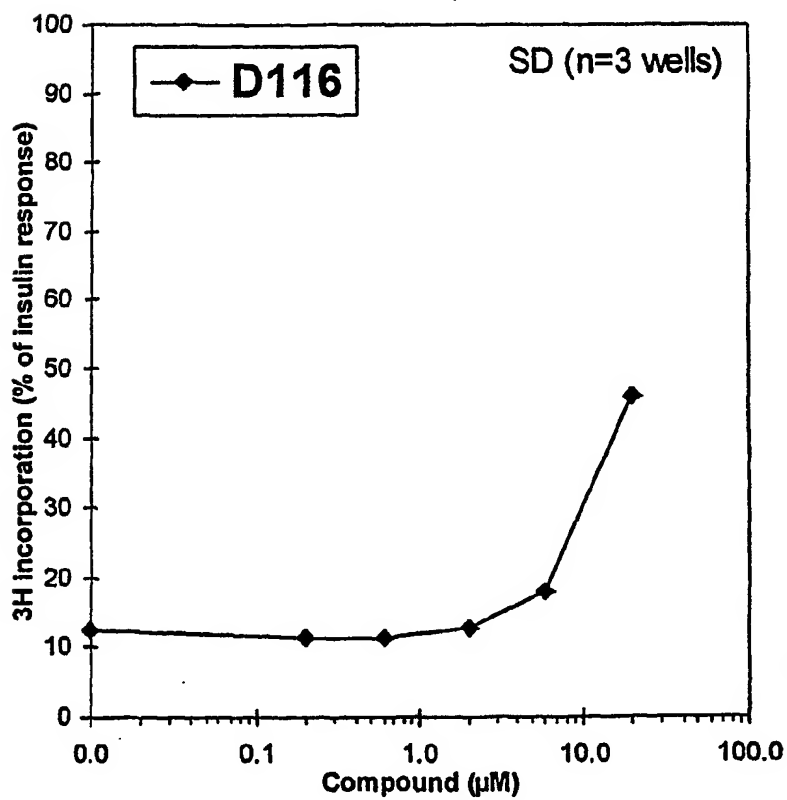


FIG. 71X

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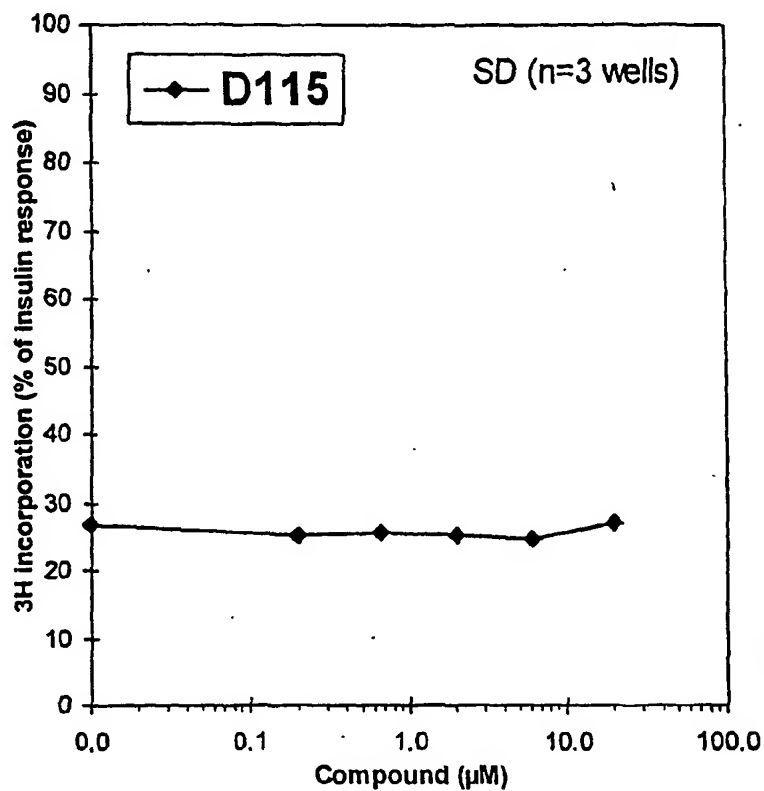


FIG. 71U

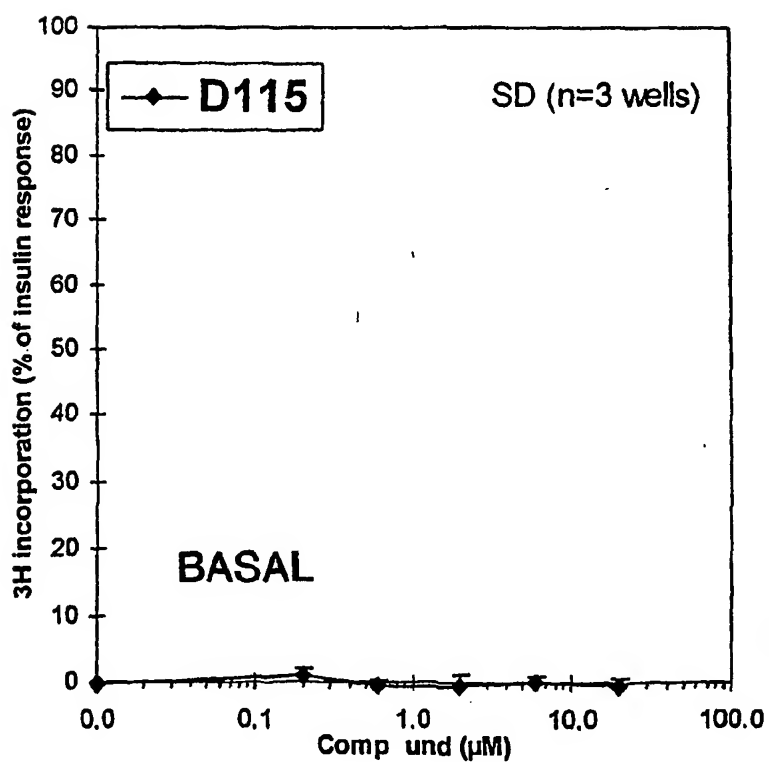


FIG. 71V

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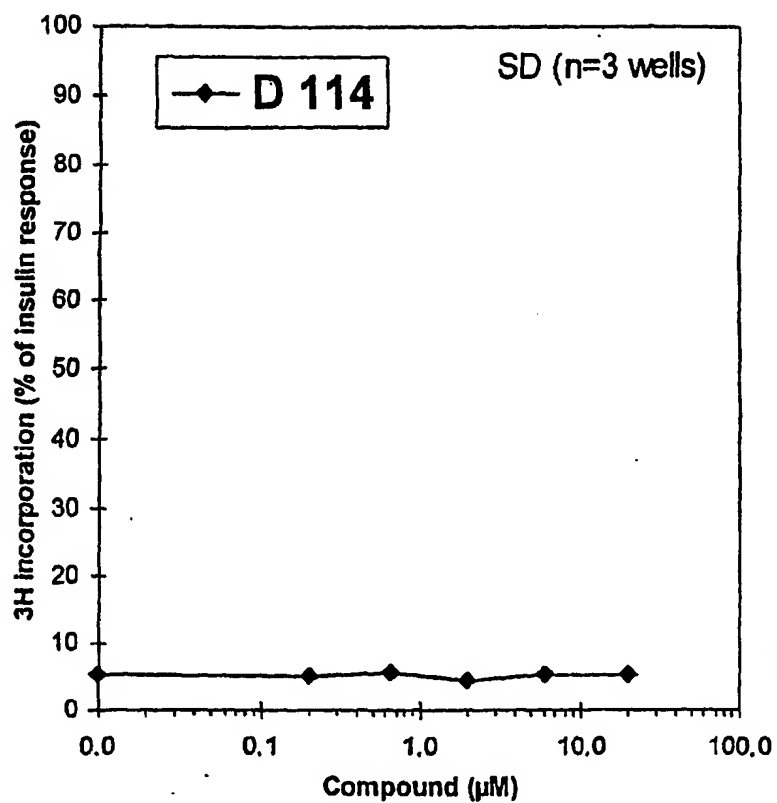


FIG. 71S

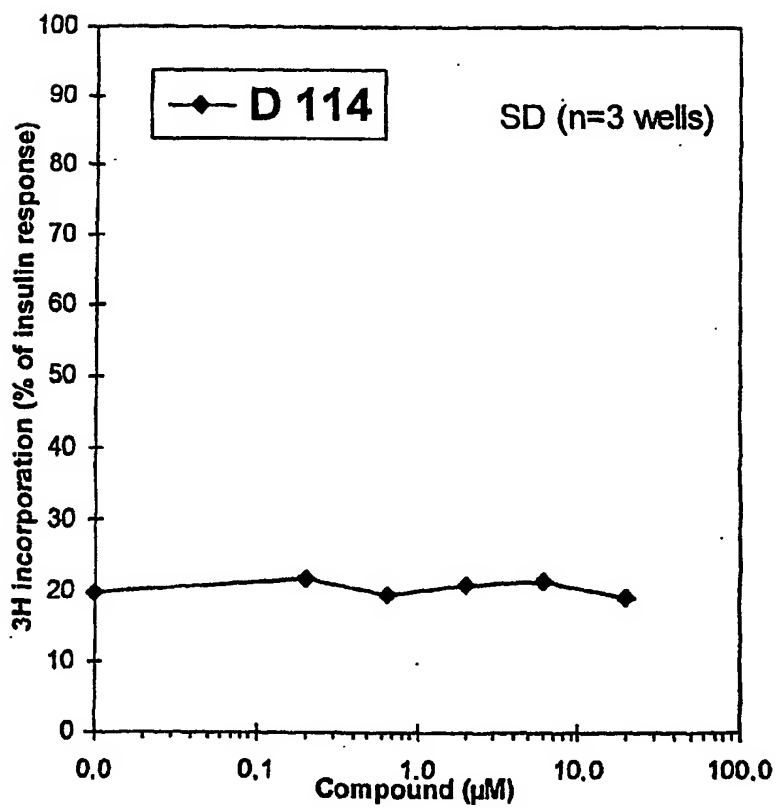


FIG. 71T

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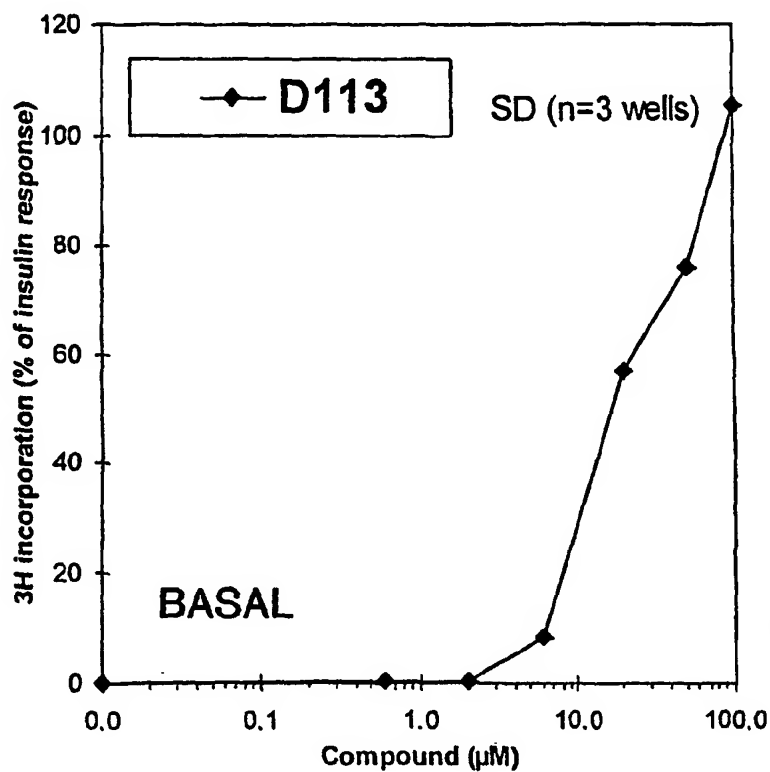


FIG. 71Q

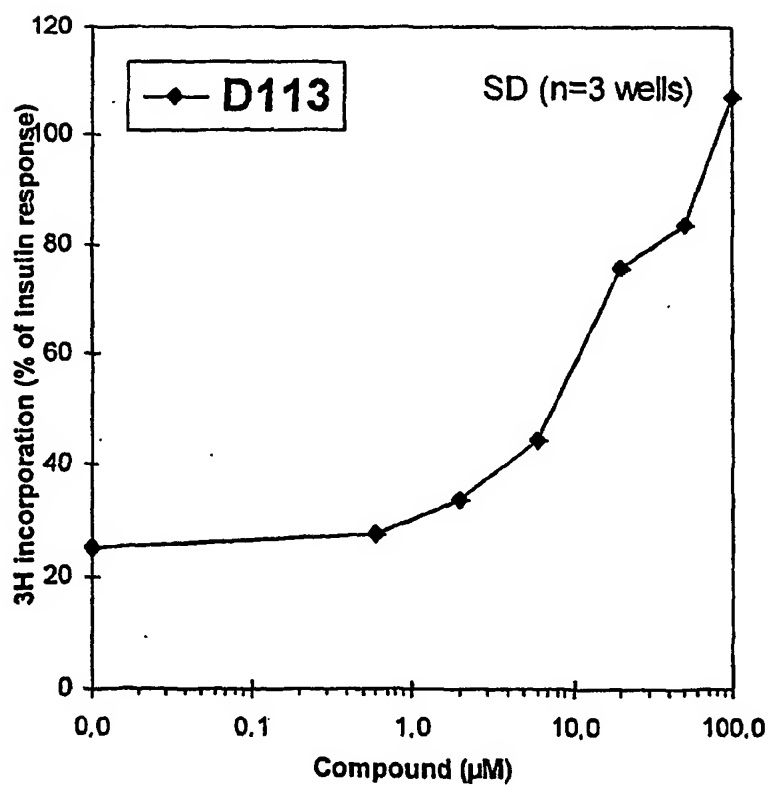


FIG. 71R

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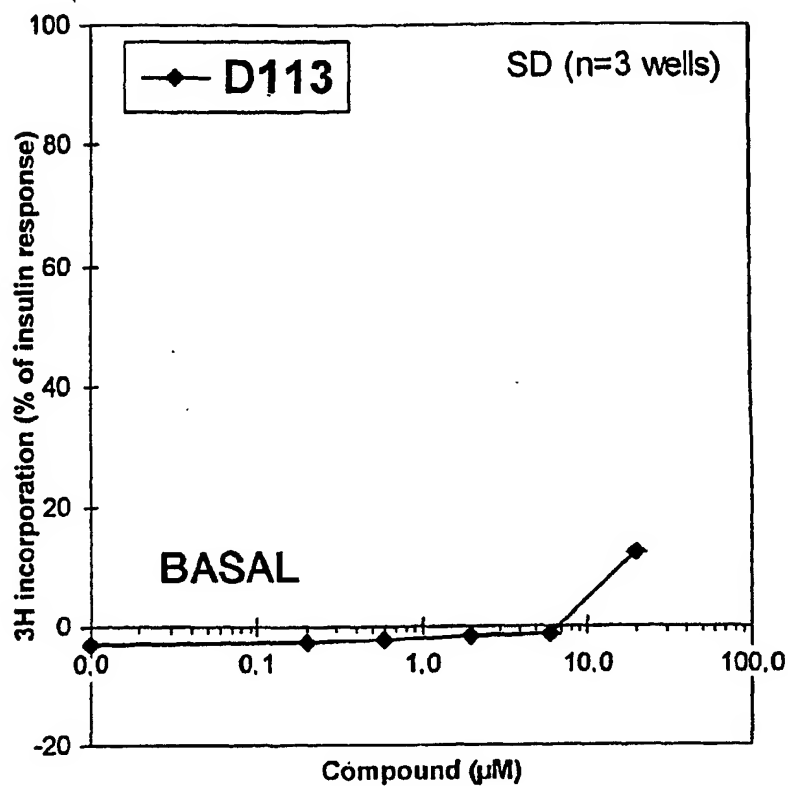


FIG. 71O

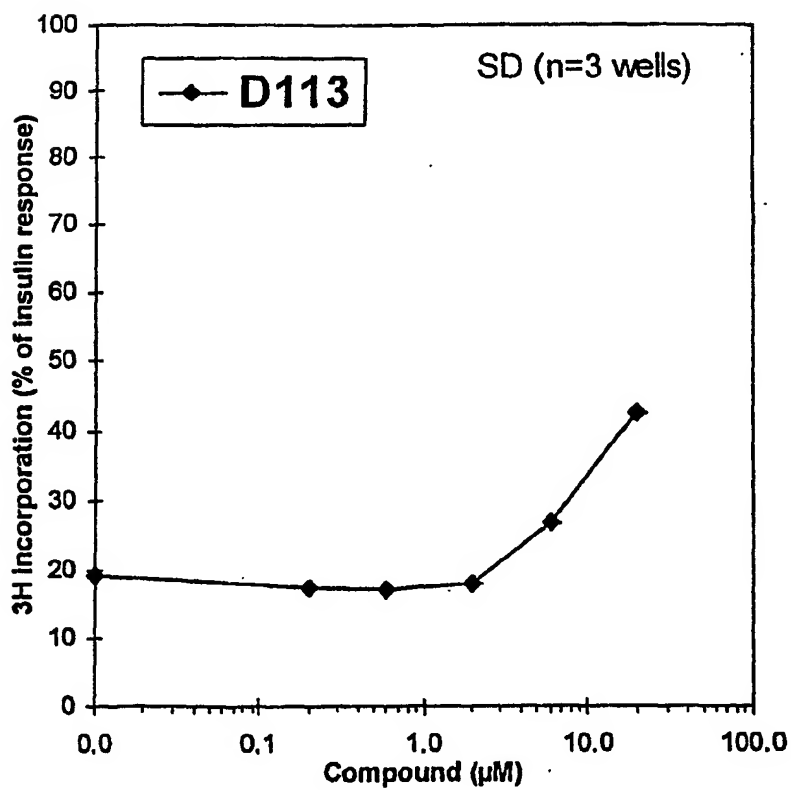


FIG. 71P

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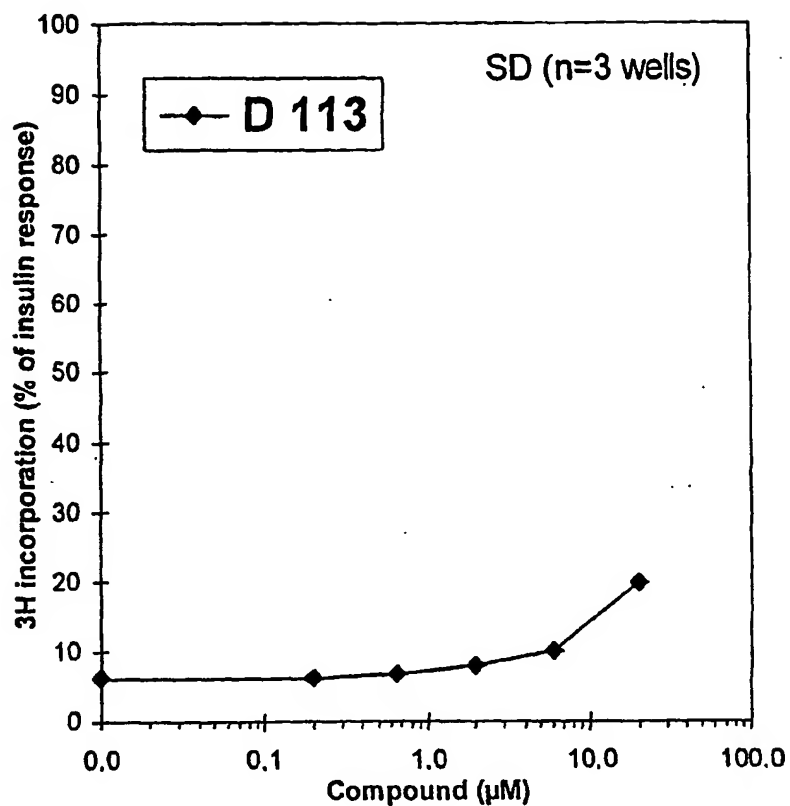


FIG. 71M

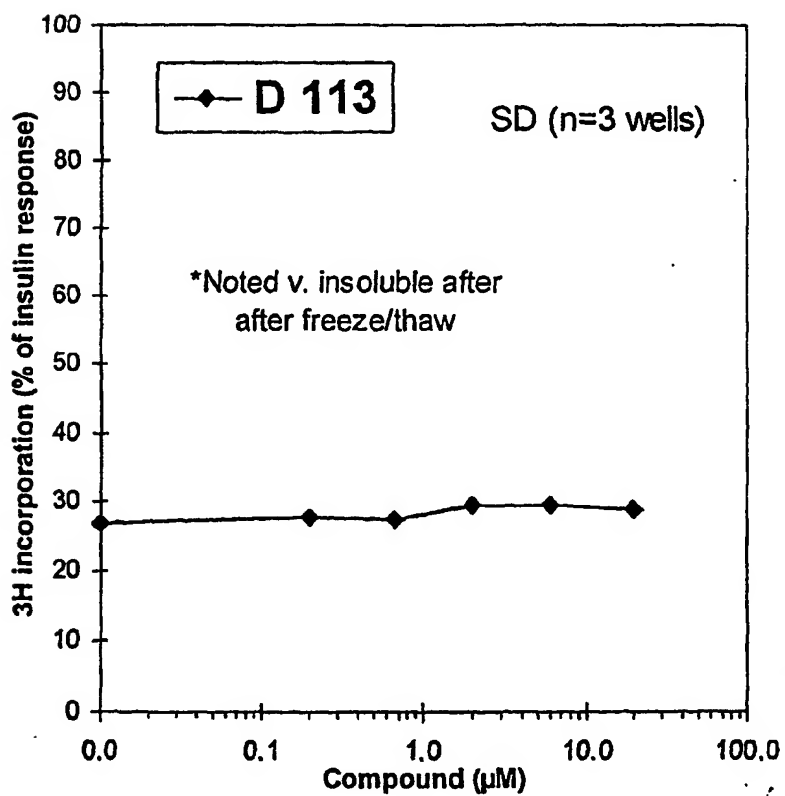


FIG. 71N

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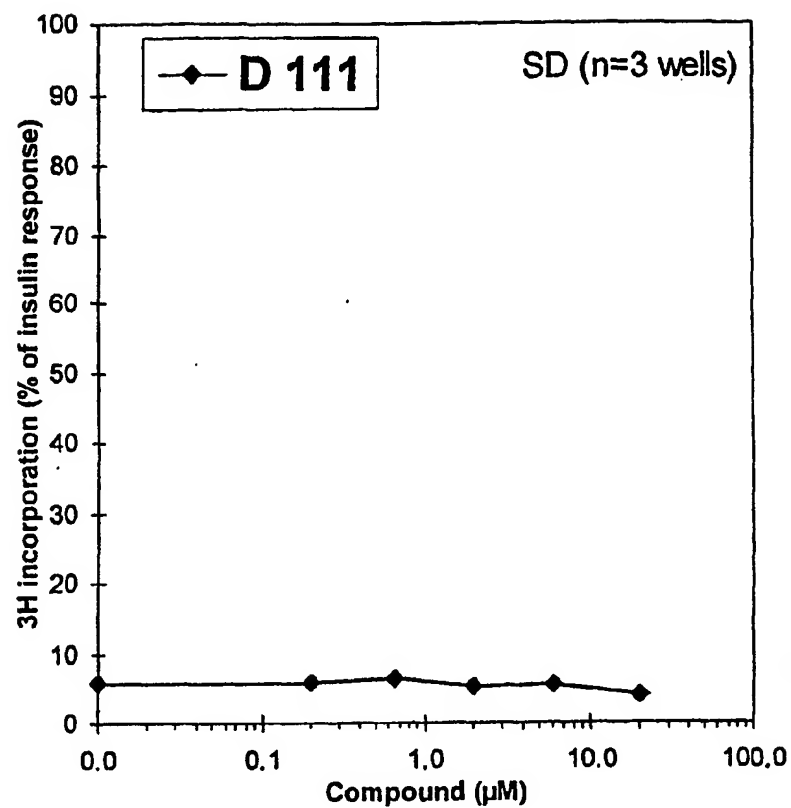


FIG. 71K

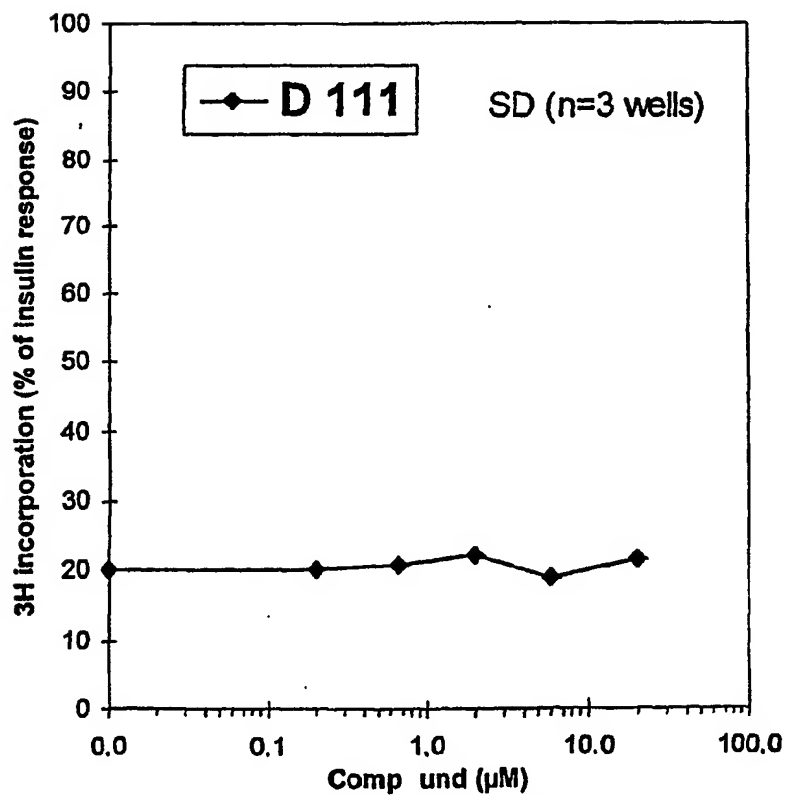


FIG. 71L

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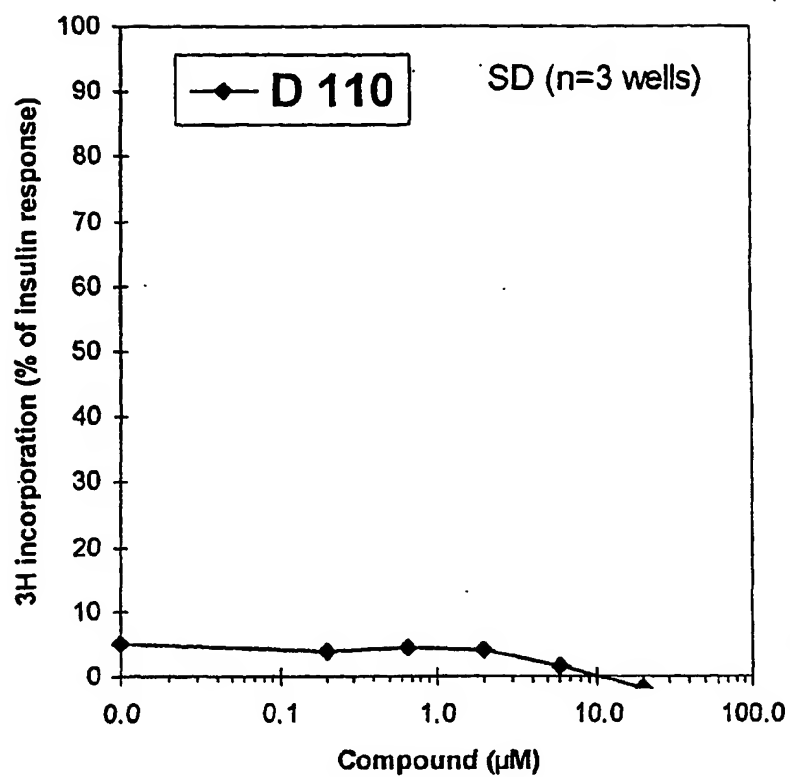


FIG. 71I

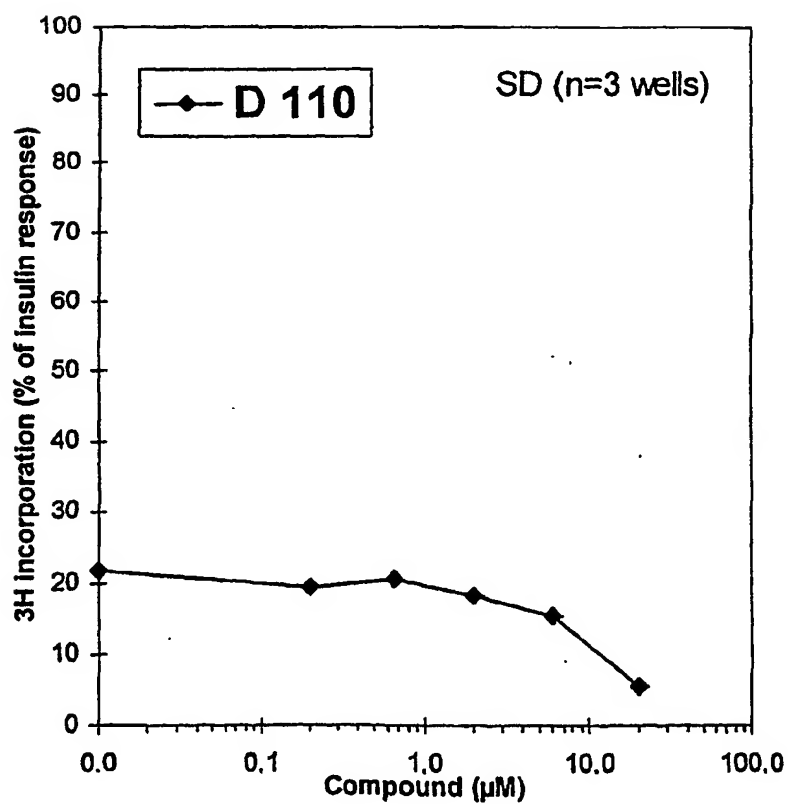


FIG. 71J

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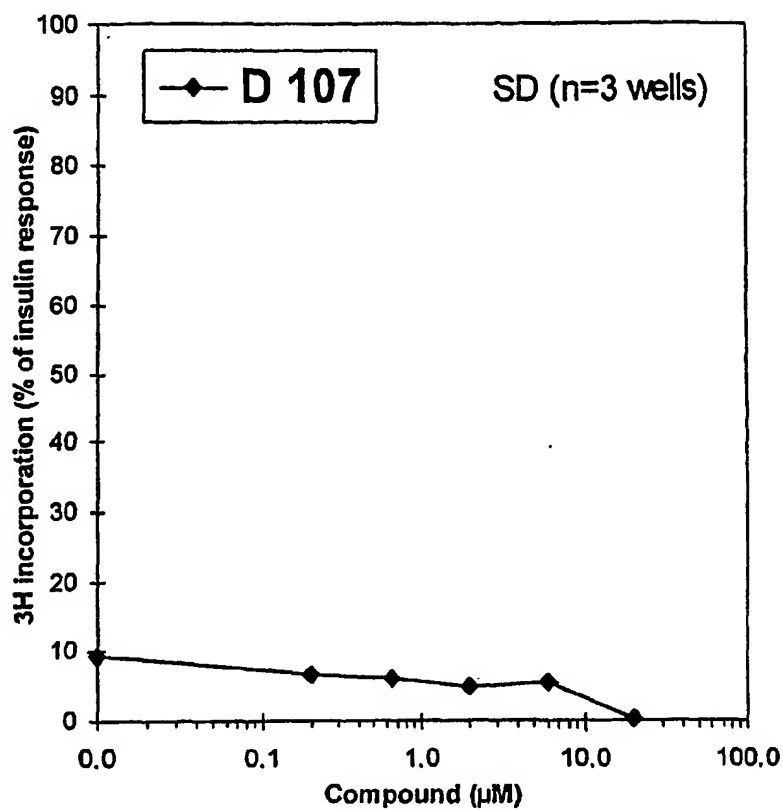


FIG. 71G

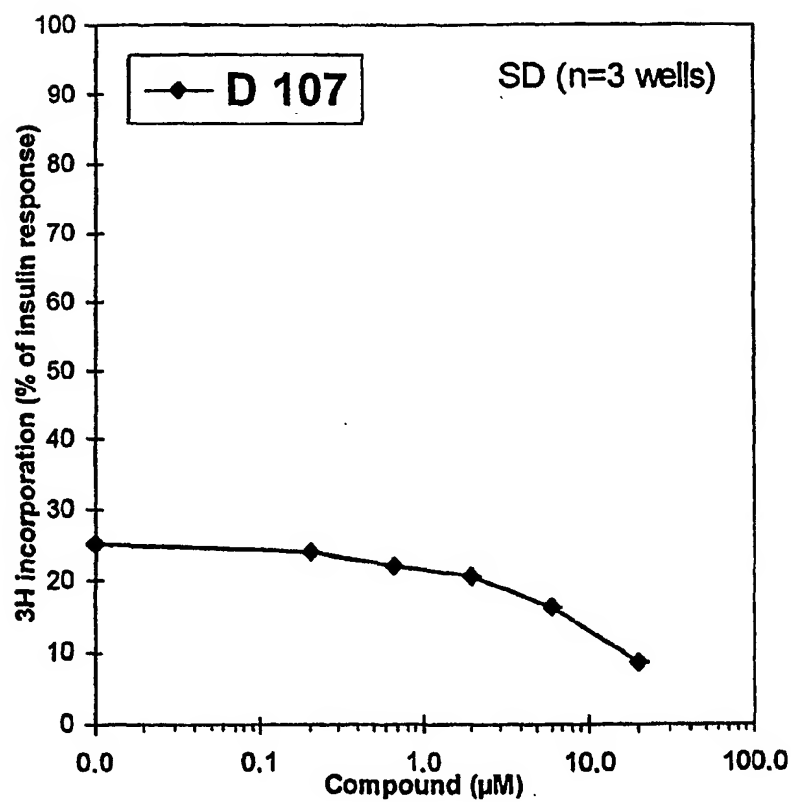


FIG. 71H

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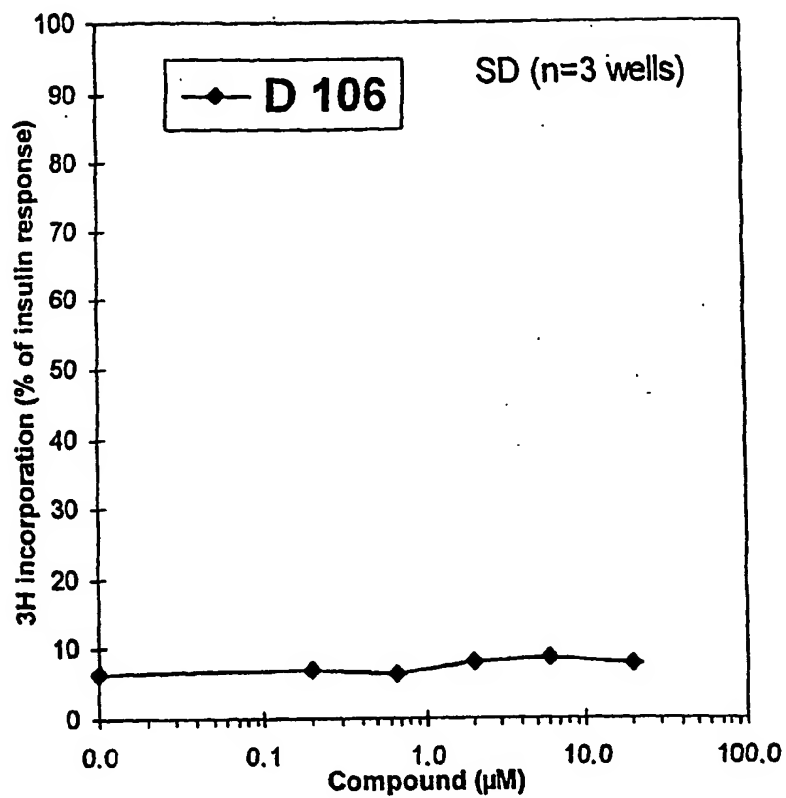


FIG. 71E

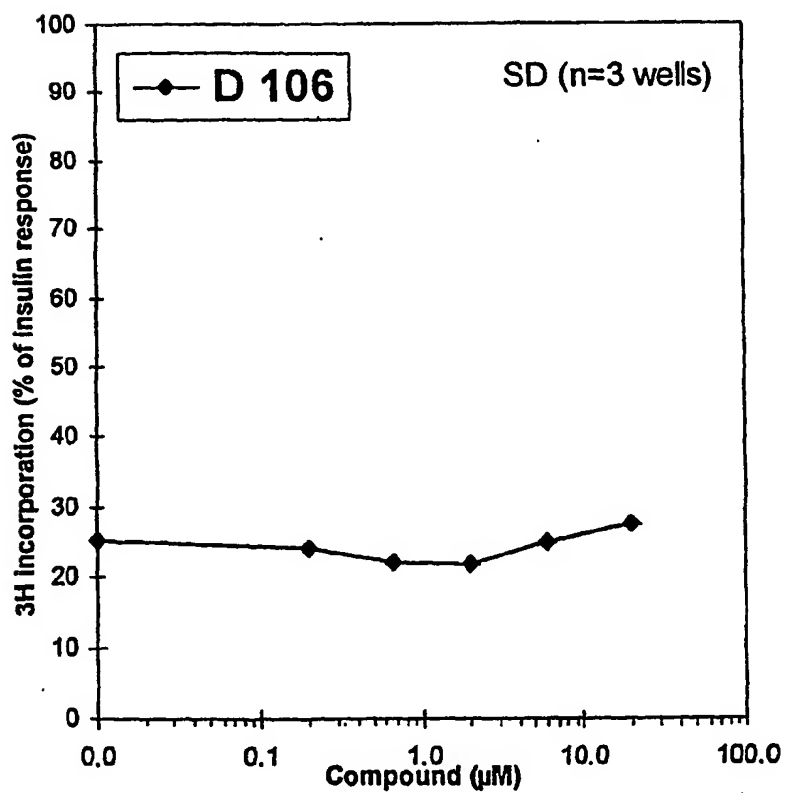


FIG. 71F

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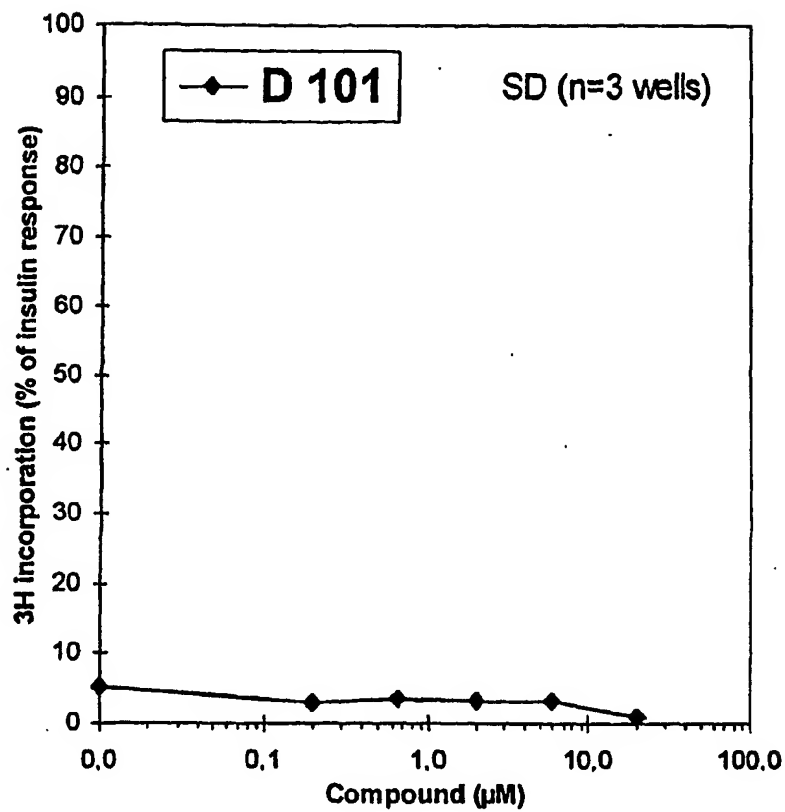


FIG. 71C

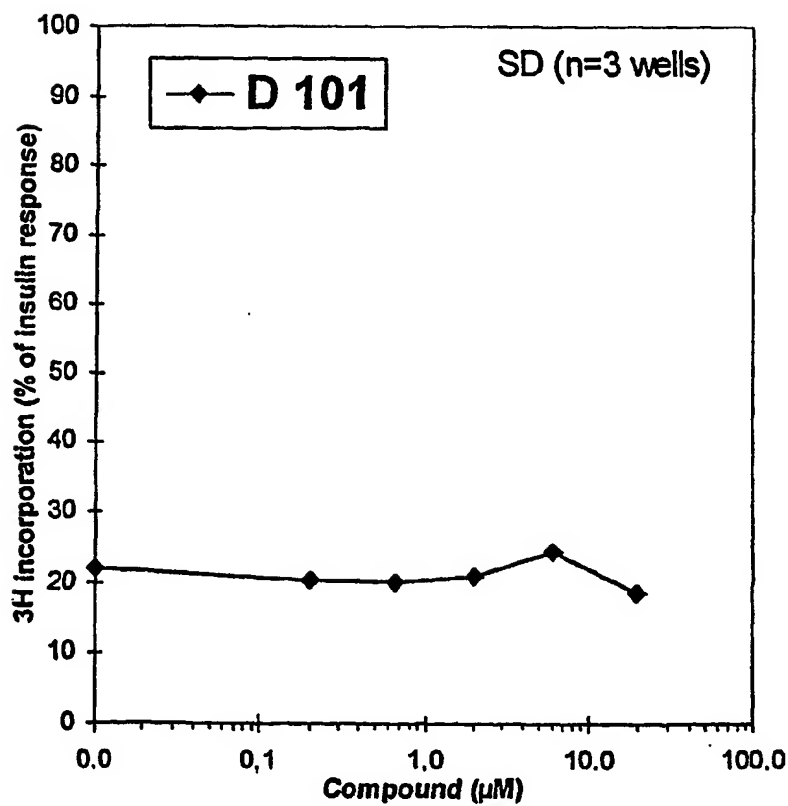


FIG. 71D

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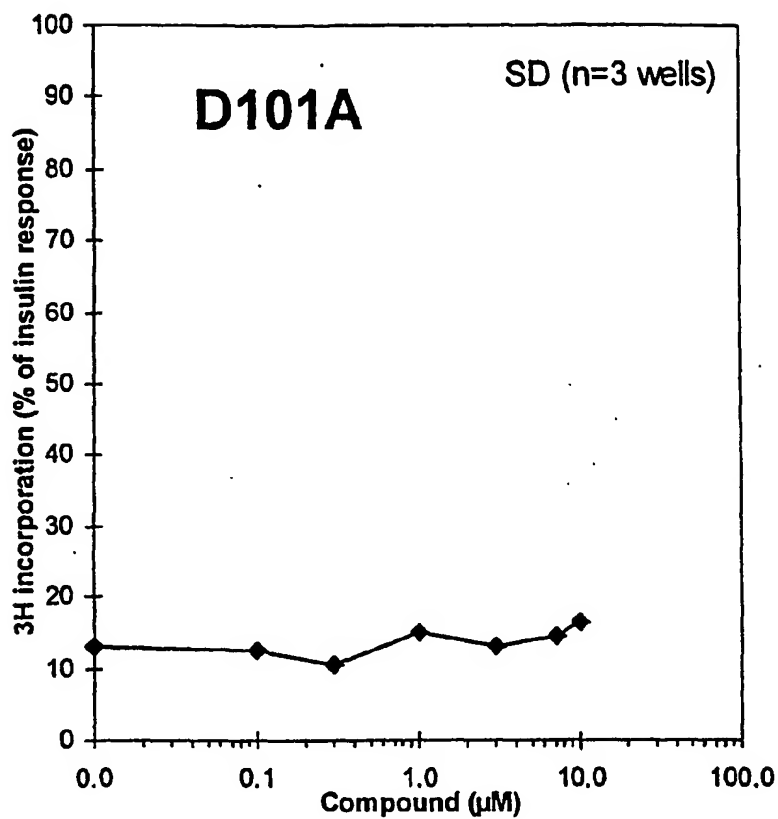


FIG. 71A

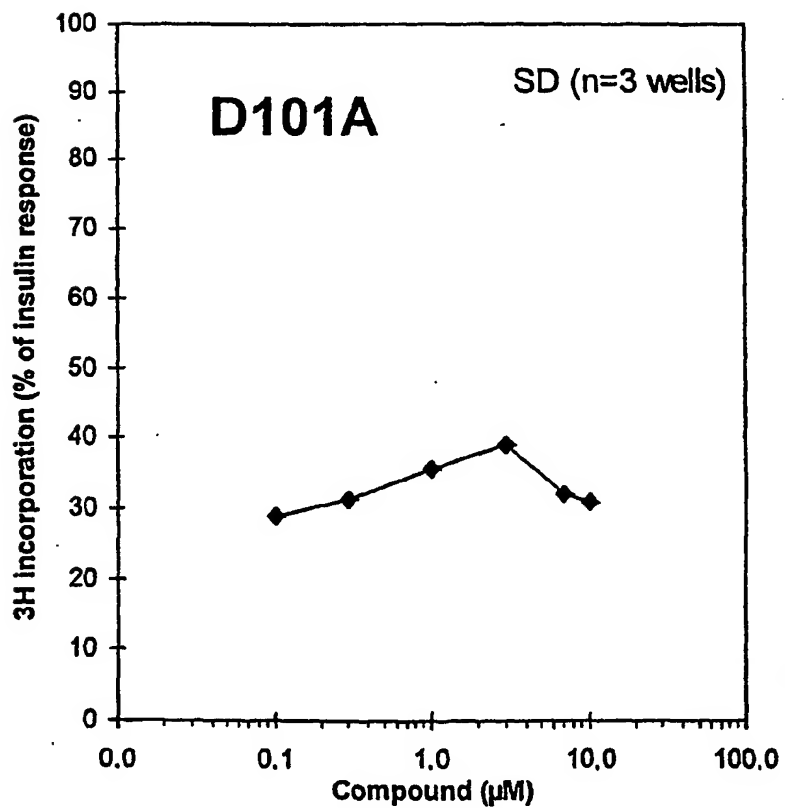


FIG. 71B

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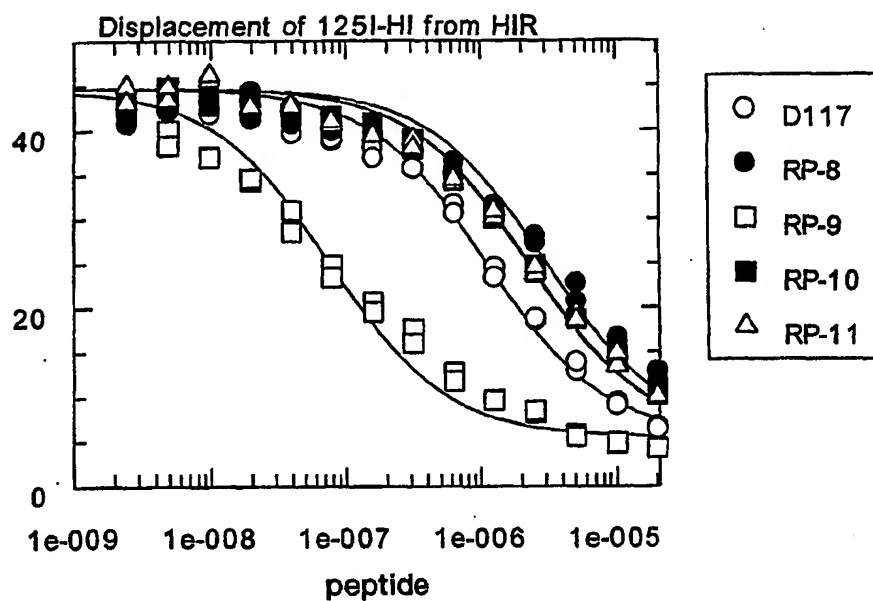


FIG. 70M

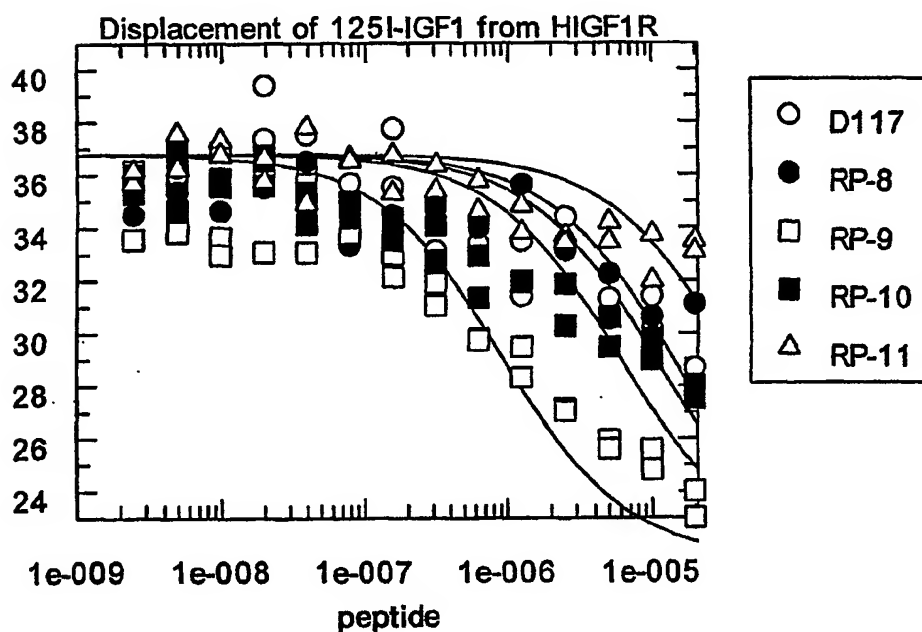


FIG. 70N

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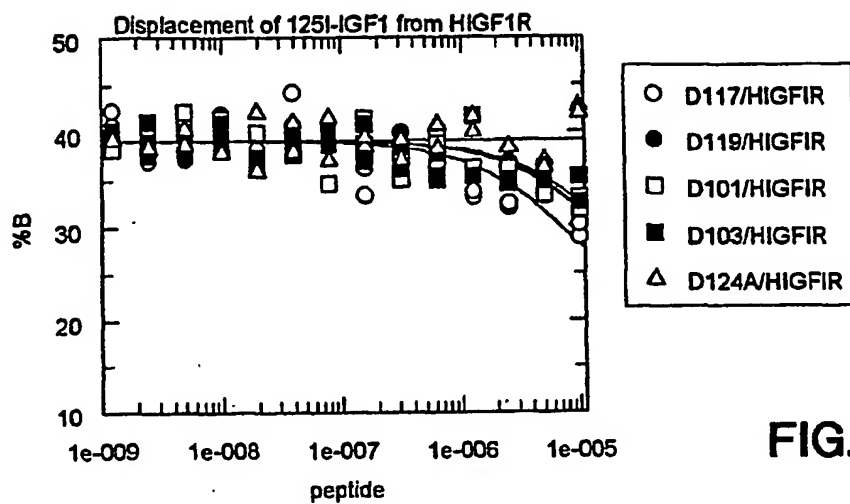


FIG. 70J

D990126A

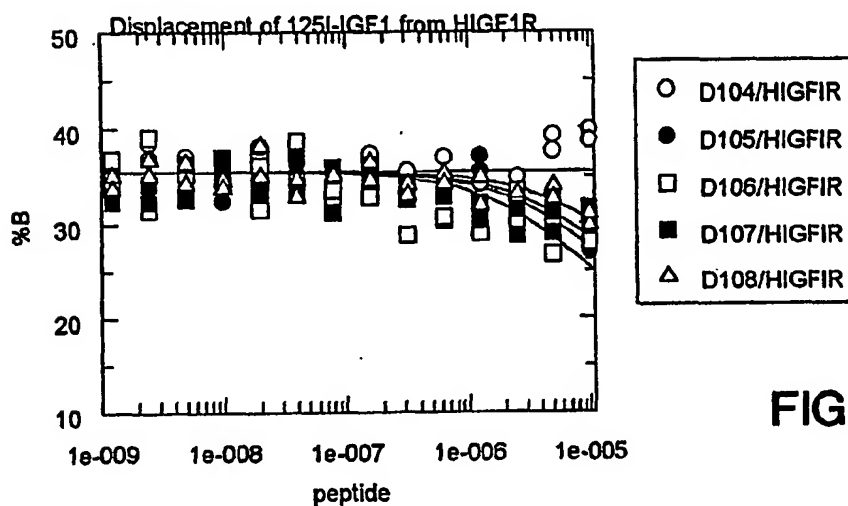


FIG. 70K

D990129A

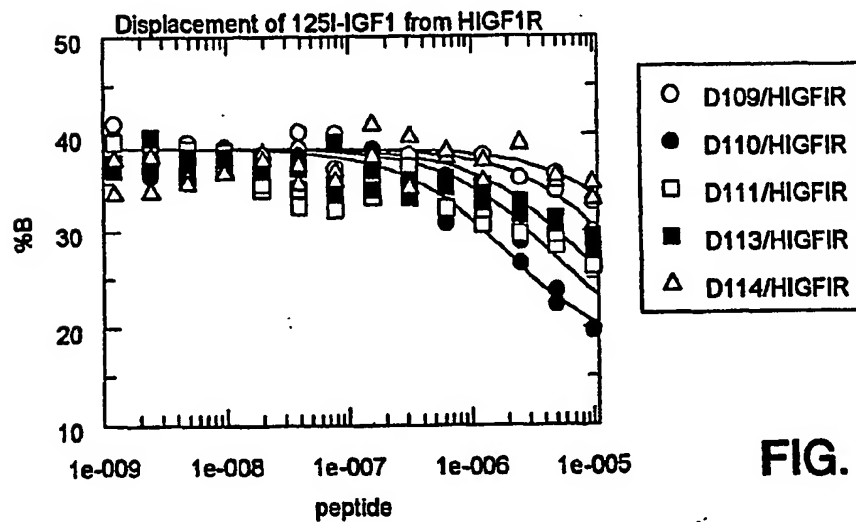


FIG. 70L

D990202A

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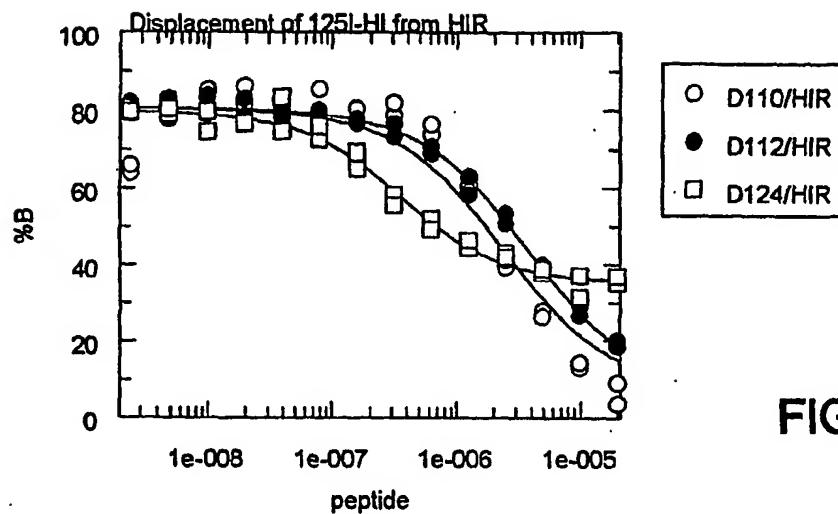


FIG. 70G

D990217A

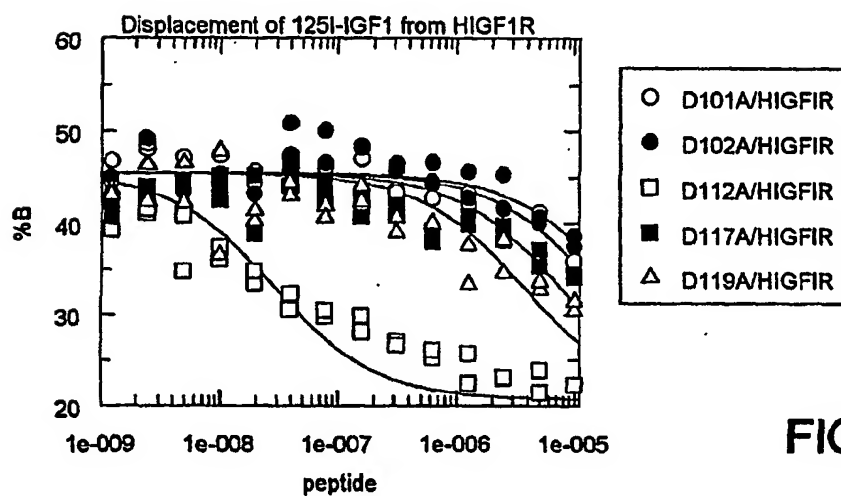


FIG. 70H

D990114A

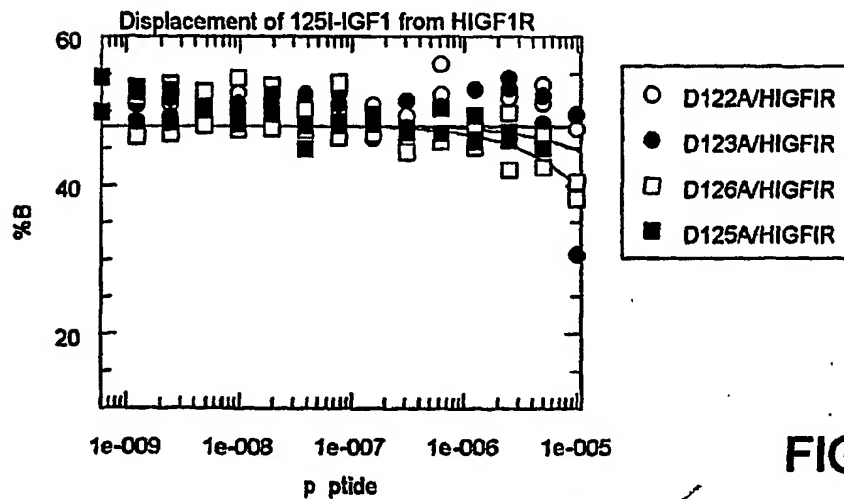


FIG. 70I

D990118A

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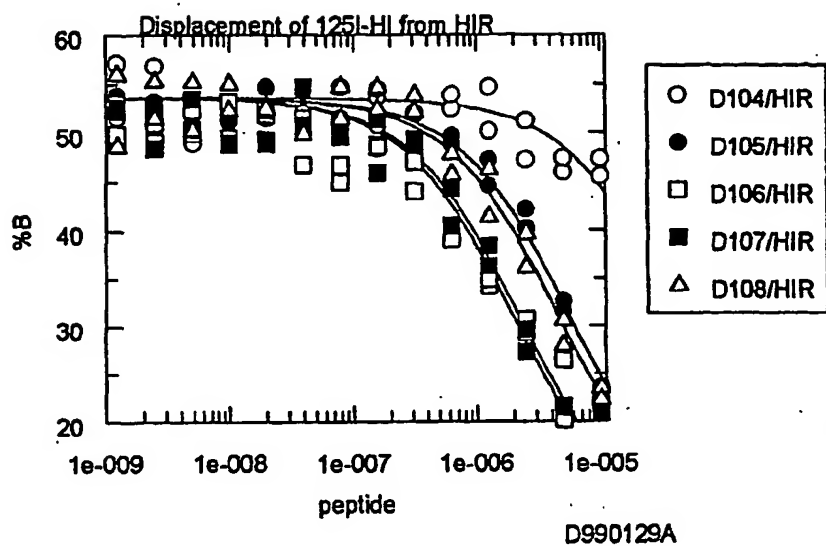


FIG. 70D

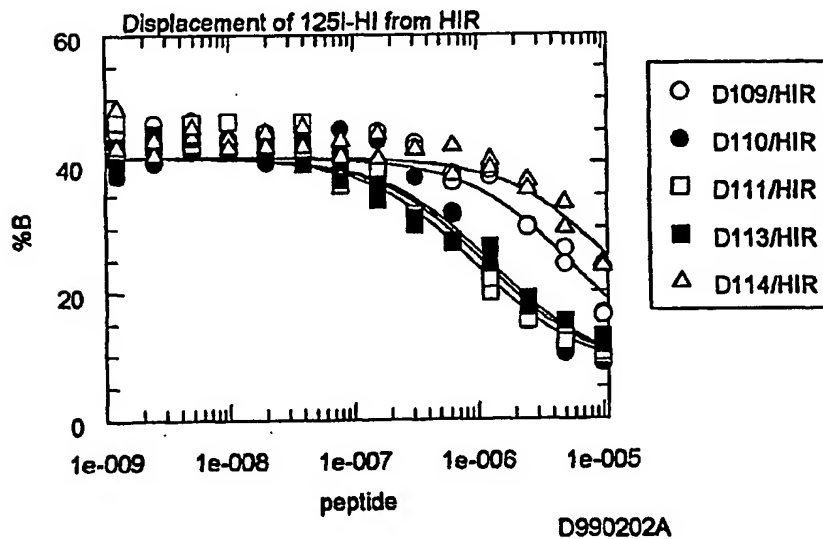


FIG. 70E

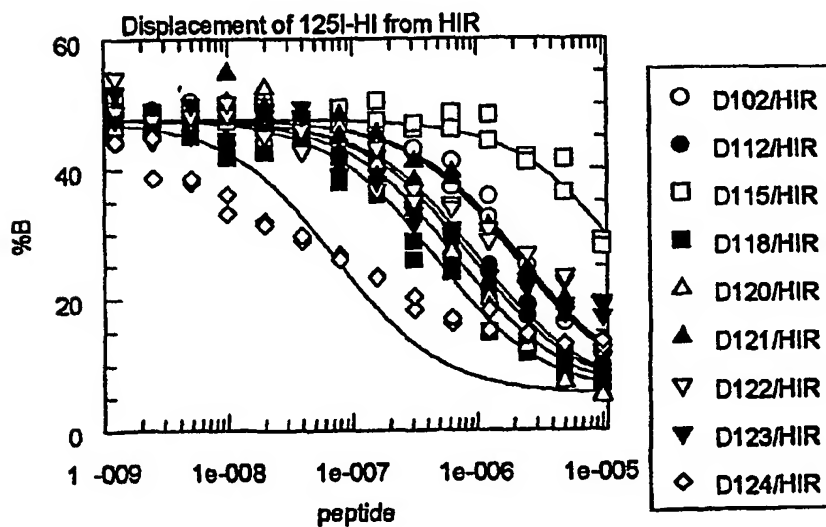


FIG. 70F

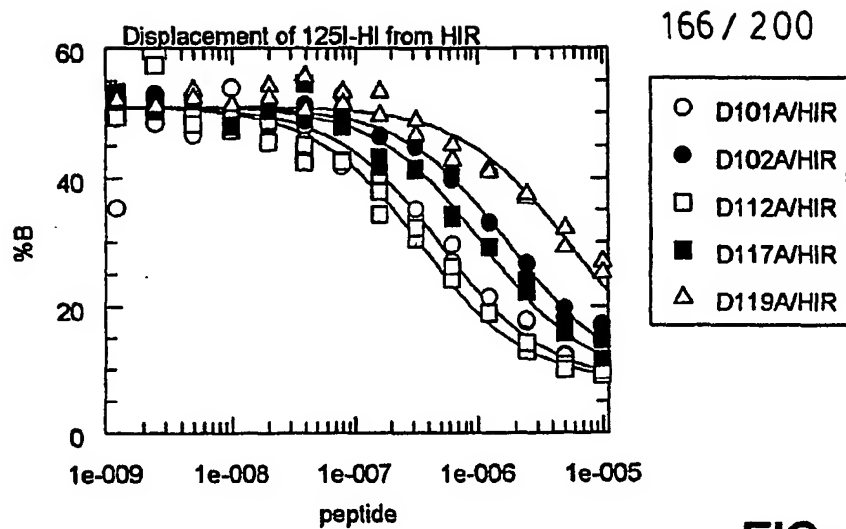


FIG. 70A

D990114A

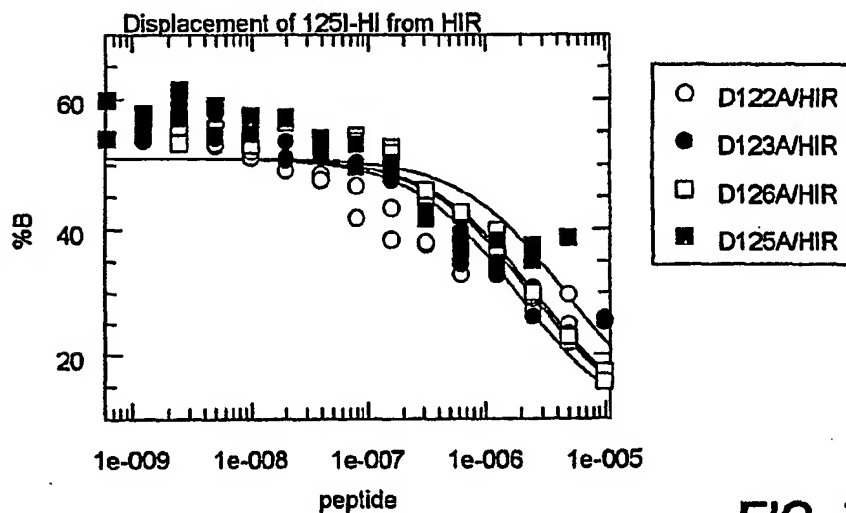


FIG. 70B

D990118A

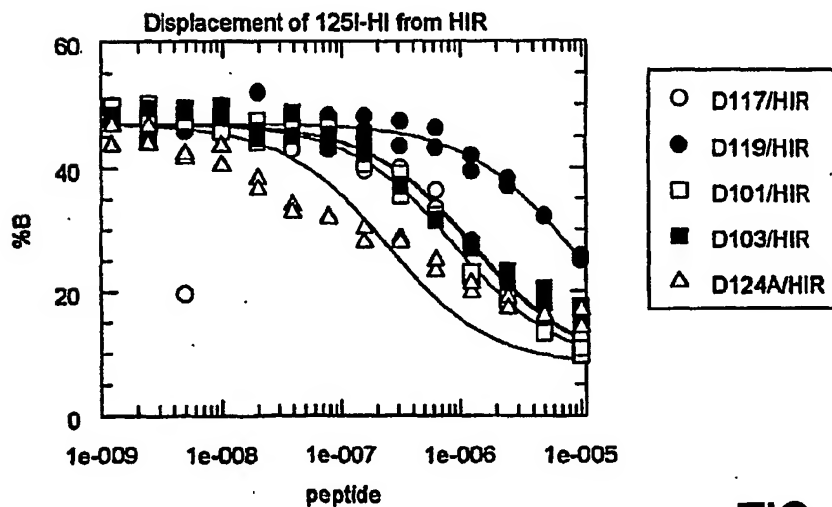


FIG. 70C

D990126A

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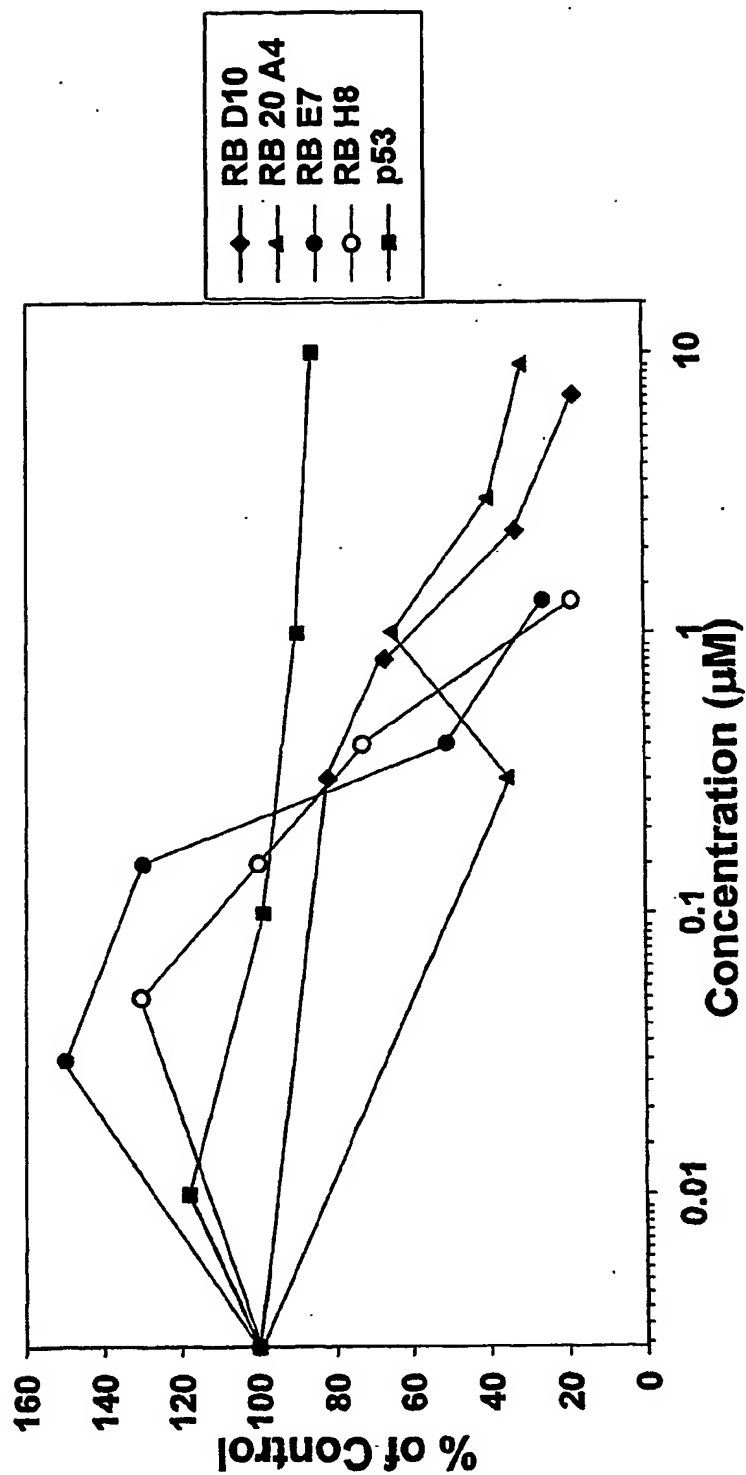


FIG. 69

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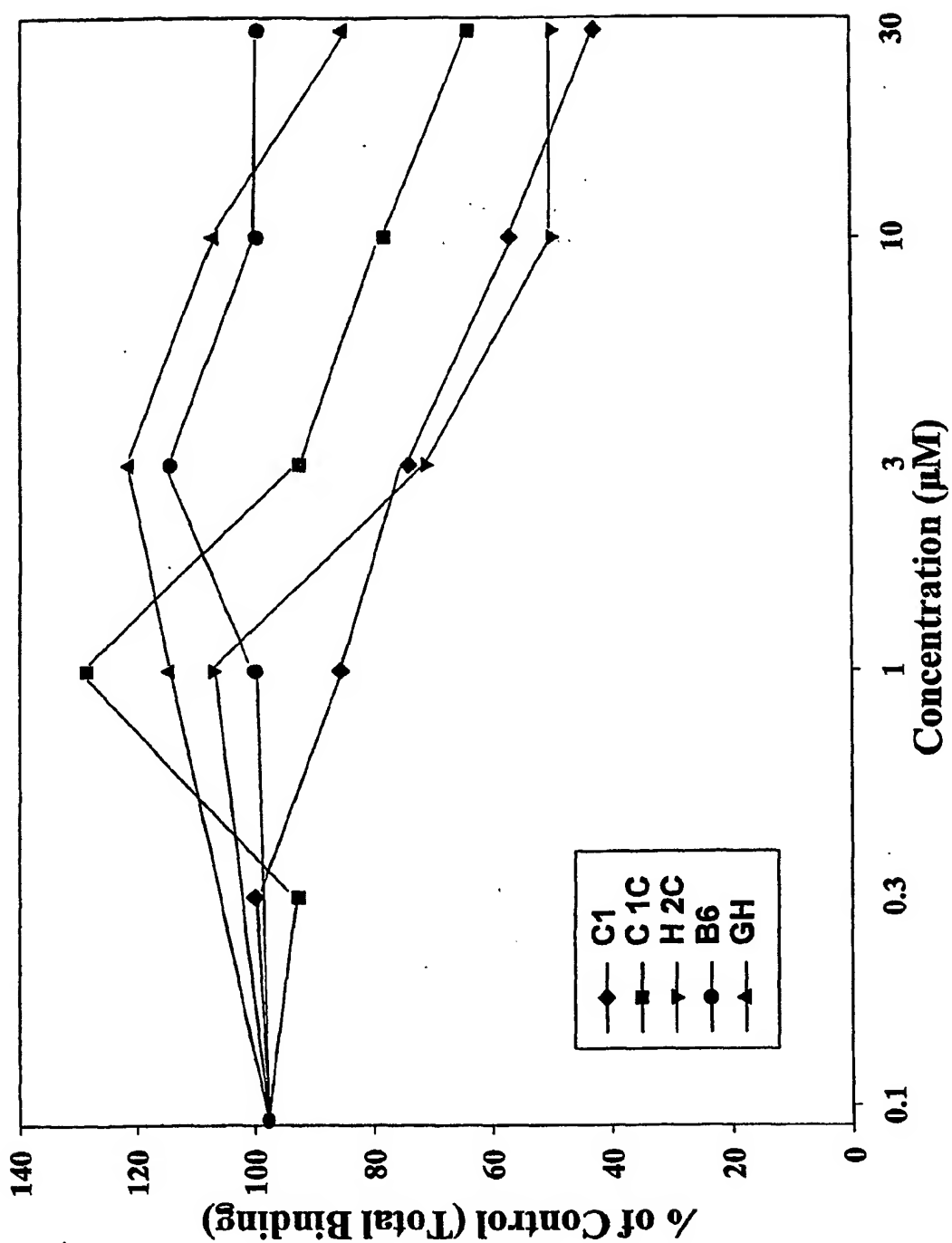


FIG. 68

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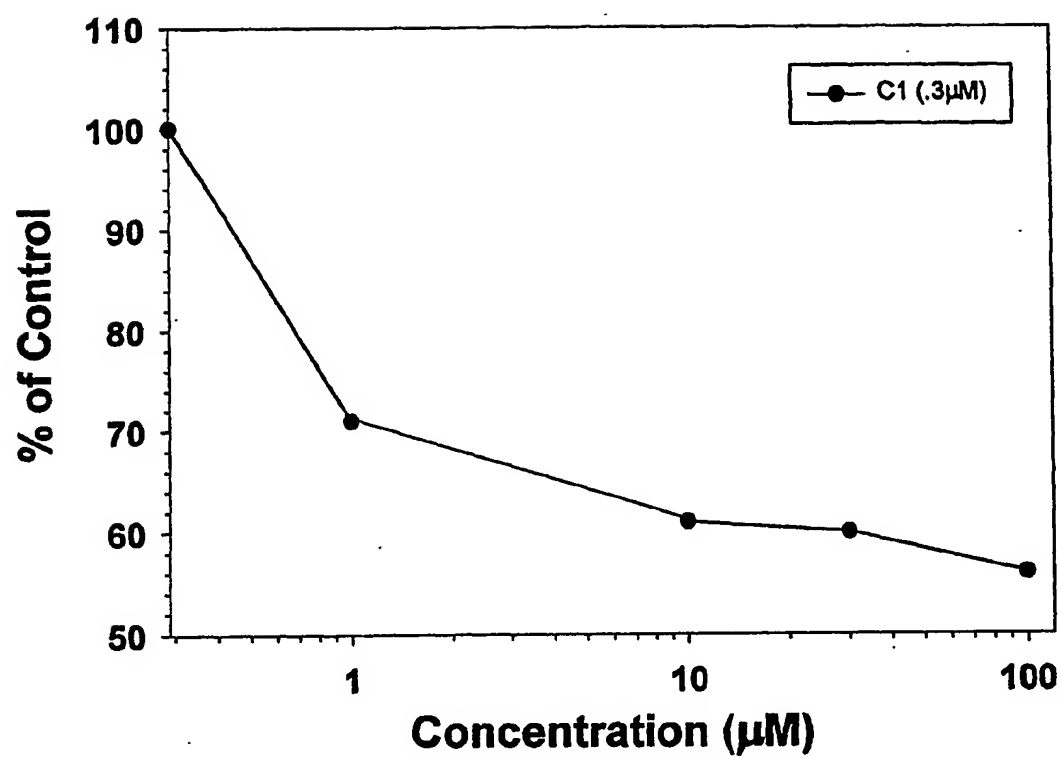


FIG. 67

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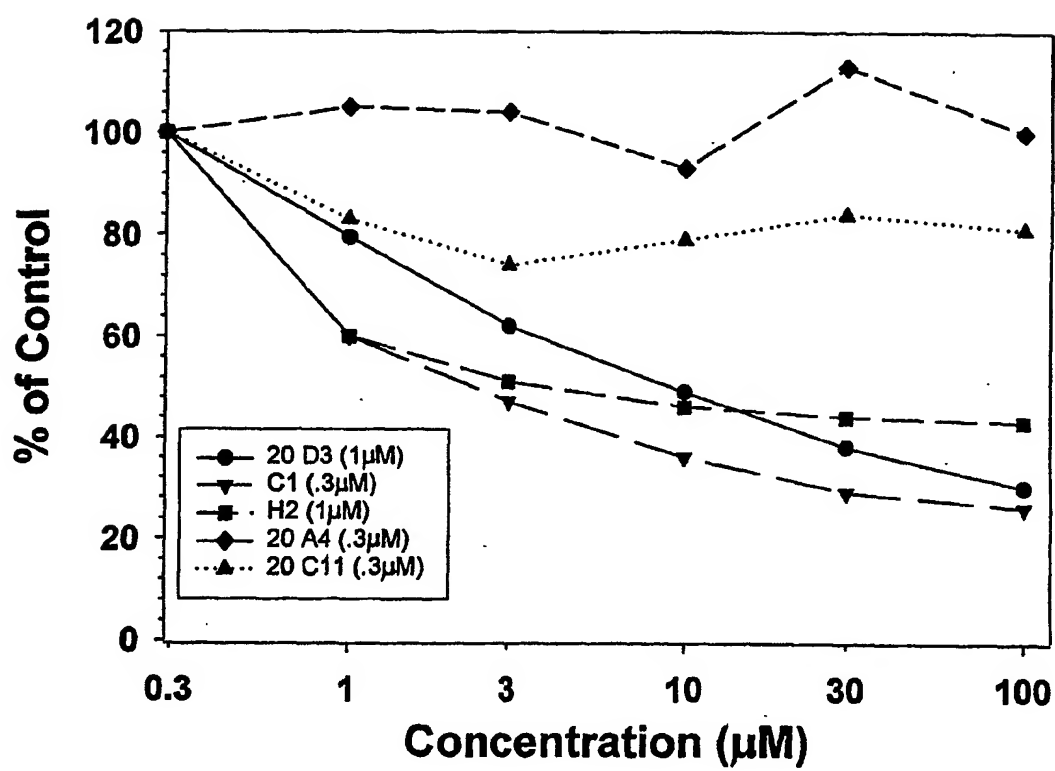


FIG. 66

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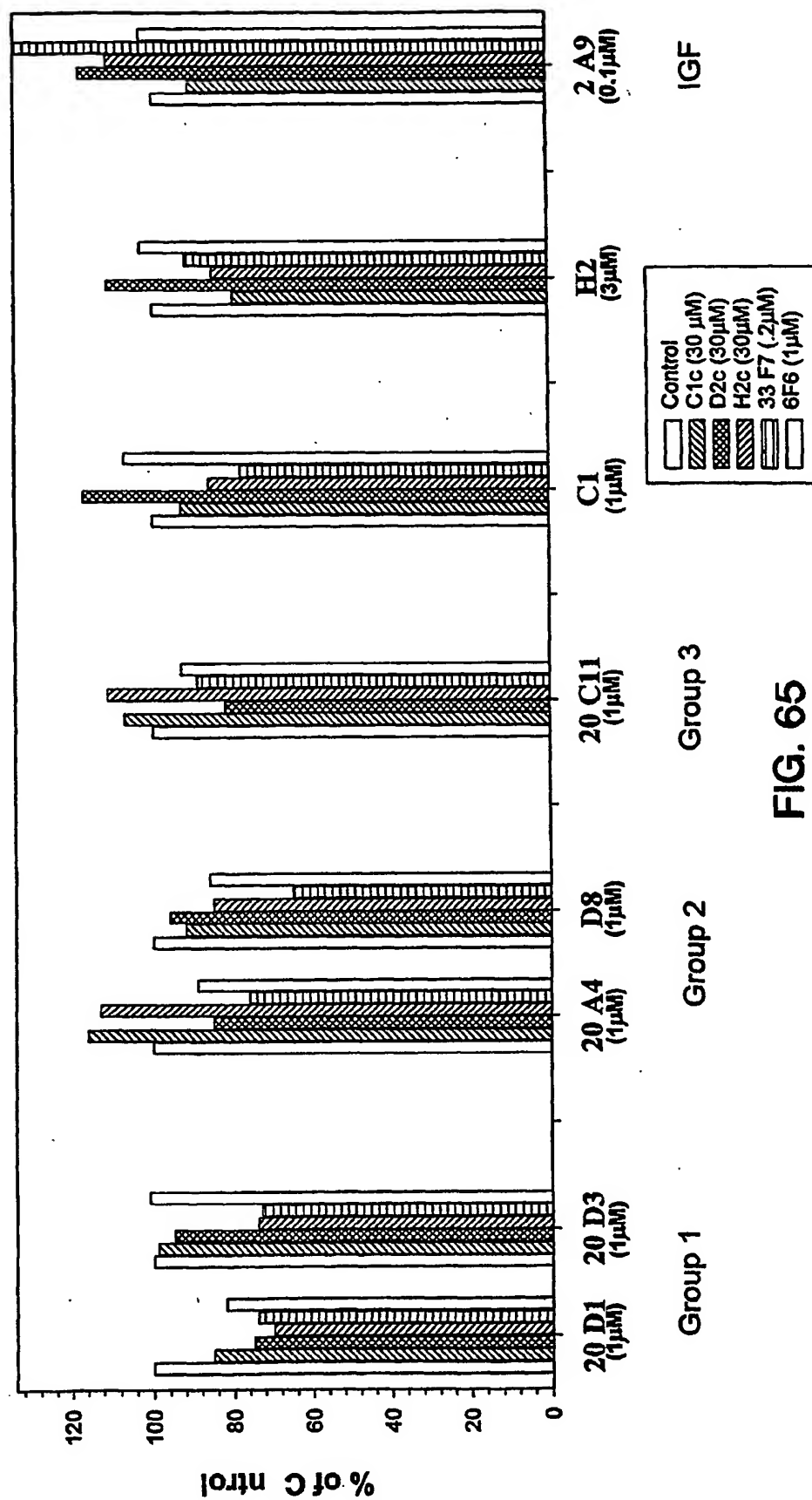


FIG. 65

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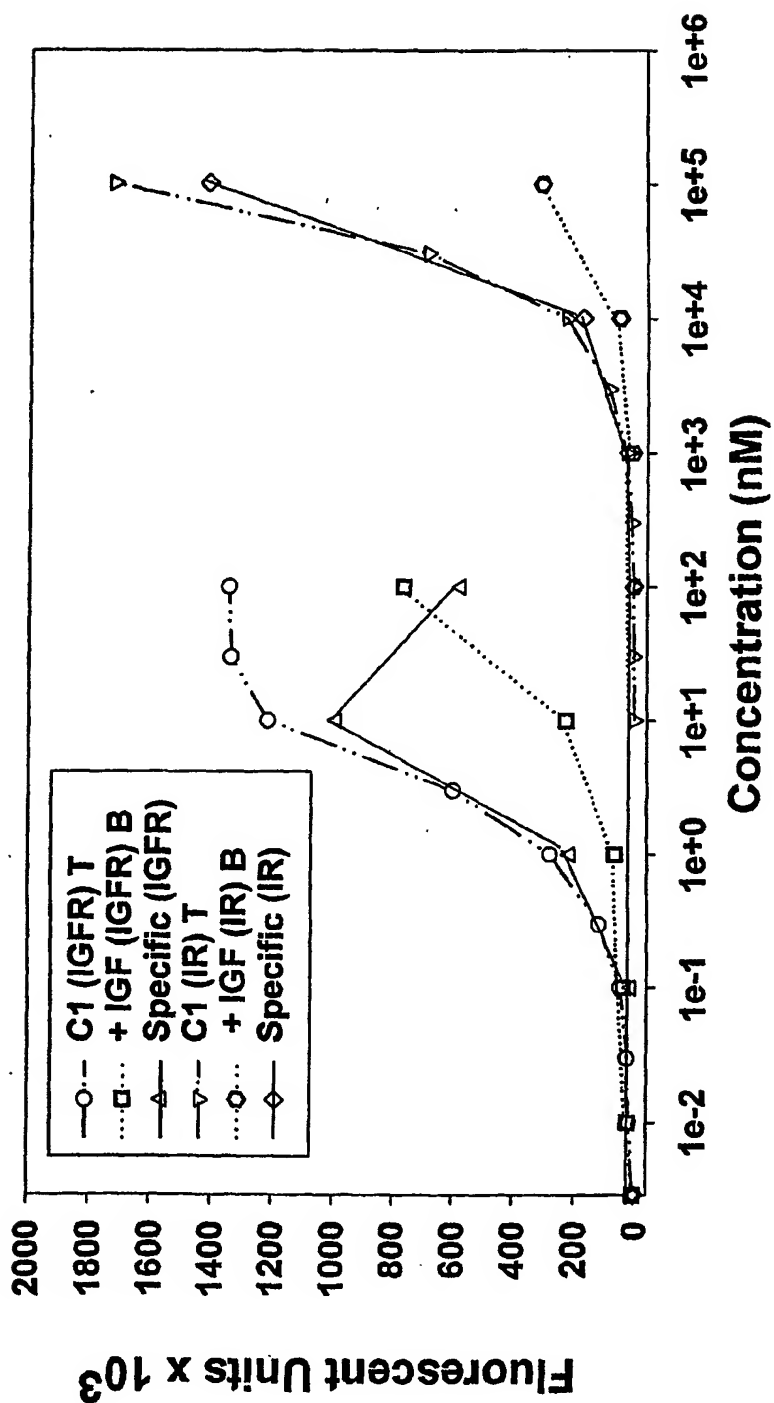


FIG. 64

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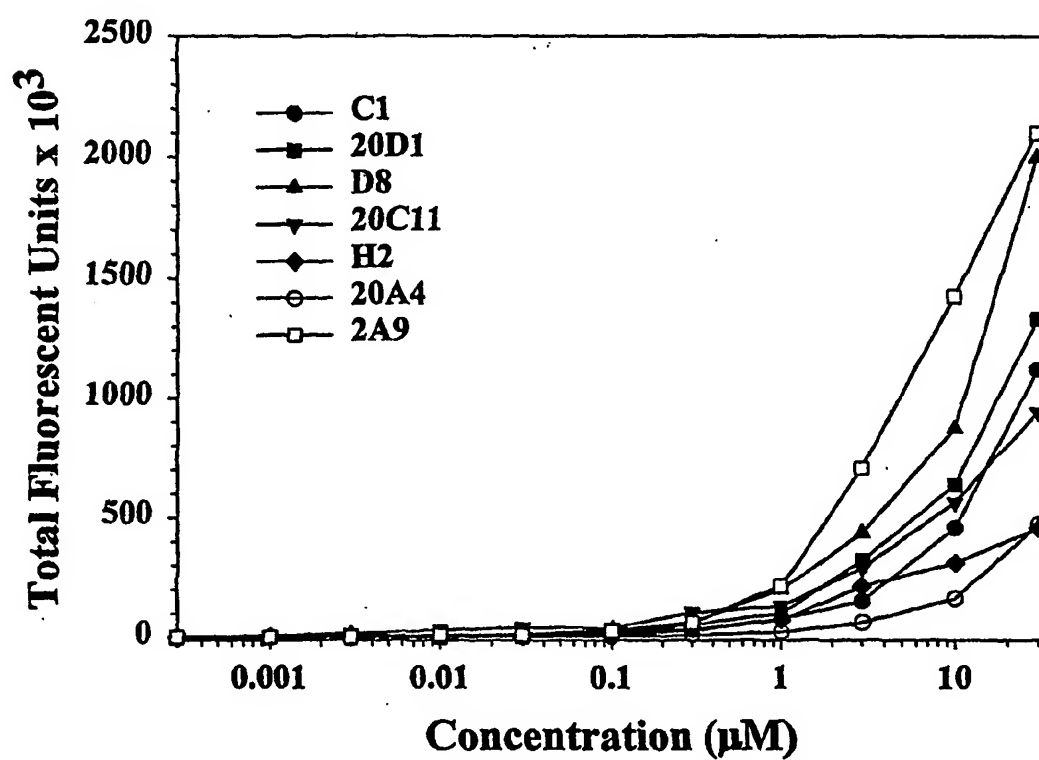


FIG. 63

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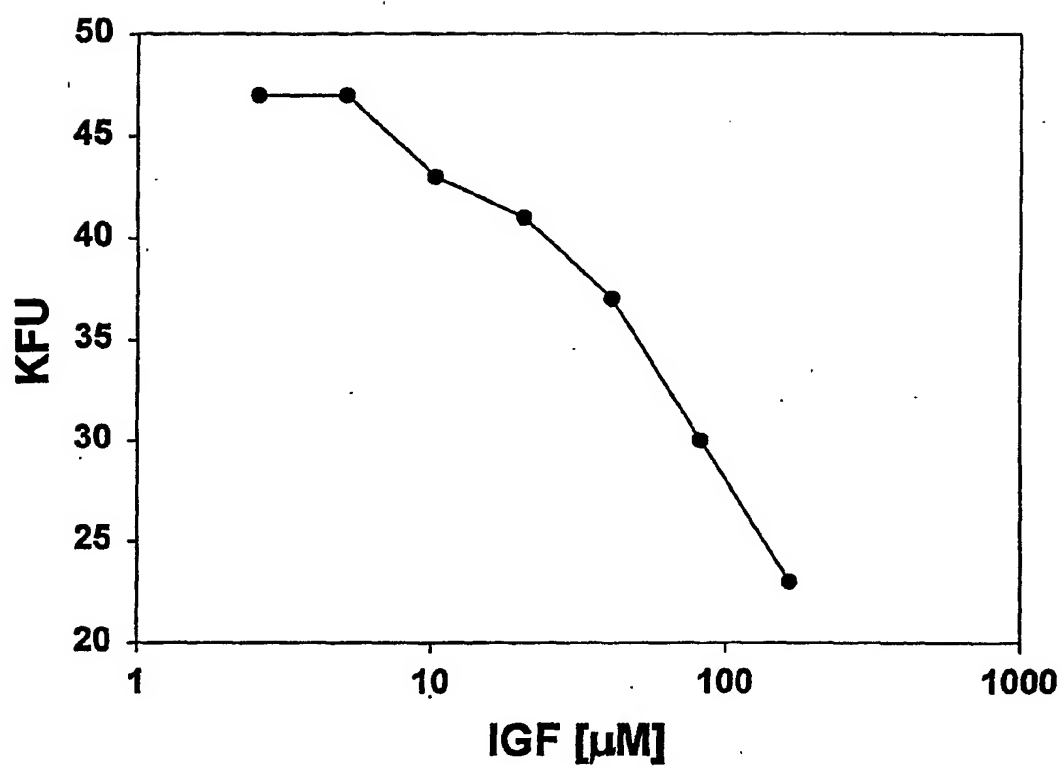


FIG. 62

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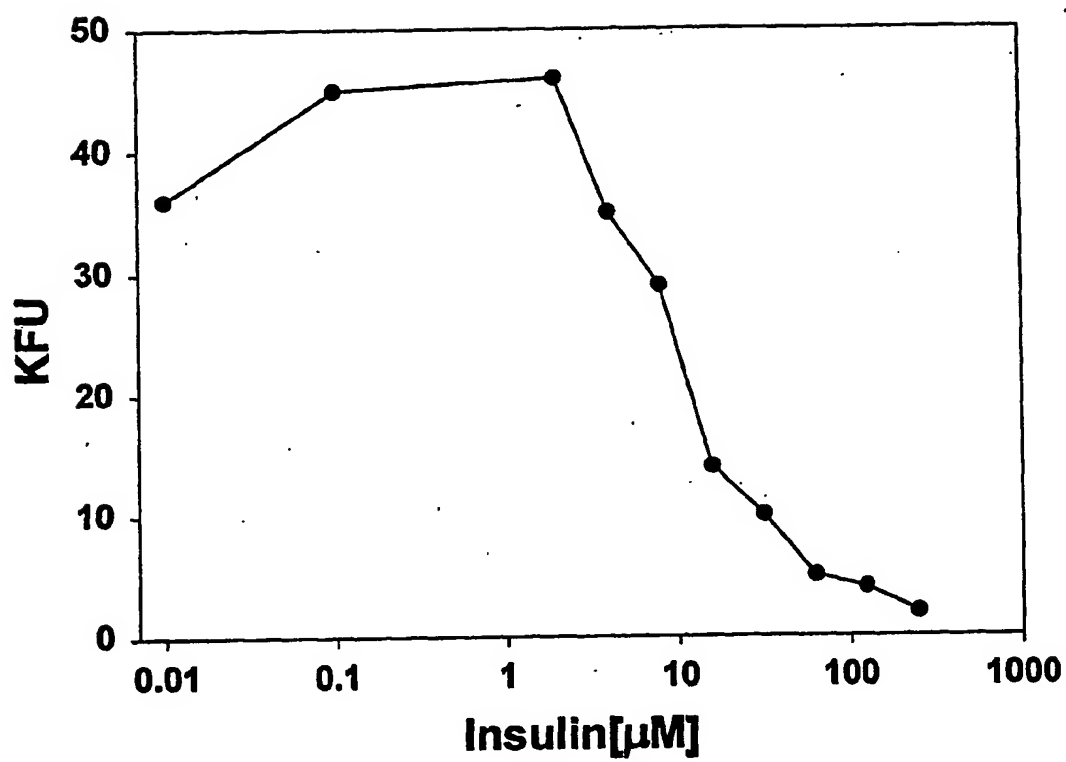


FIG. 61

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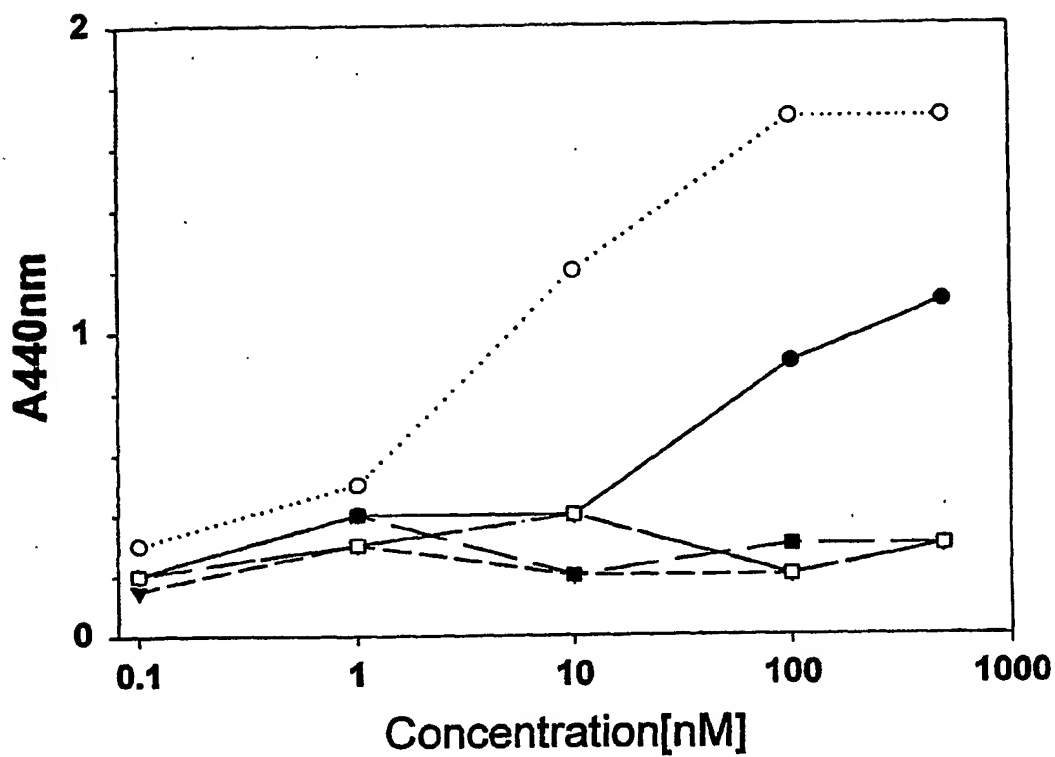


FIG. 60

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	CLONES	VHCDR3	
	%Inhibition Activity		
	118:	PFFV <u>FYRGQDT</u>	54%
?	InsulB:	FVNQH <u>L</u> CGSH <u>L</u> VEALY LV <u>CG</u> ERGFFYTPKT	
	12H10:	C VVYNYA <u>G</u> <u>RG</u> T	42%
Ang?	13-e-4:	VQAWDGL <u>G</u> RES	52%
?	13h9:	GGL <u>G</u> RRDWL	30%
?	24:	GGRR H RLG	
	InsulinA	<u>GIVEQCCT</u> SIC <u>SLYQ</u> <u>LENYCN</u>	
	11a8:	<u>ENYGNSE</u>	32%
?		GDQELQNY	None
N/A			

FIG. 59

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GGCCATCCGG CCATGGCCGA GGTGCAGCTG TTGGAGTCTG GGGGAGGCTT GGTAAAGCCT	60
E V Q L L E S G G G L V K P	
GGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG	120
G G S L R L S C A A S G F T F S N A W M	
AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAGCAAA	180
S W V R Q A P G K G L E W V G R I K S K	
ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA	240
T D G G T T D Y A A P V K G R F T I S R	
GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC	300
D D S K N T L Y L Q M N S L K T E D T A	
GTGTATTACT GTACCACATA CGATTAGTGG GGGGTCTTGG TGGTCTGGGG TCAAGGAACT	360
V Y Y C T T Y D W G V L V V W G Q G T	
CTGGTCACCG TCTCCTCAGG TGGAGGCGGT TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA	420
L V T V S S G G G G S G G G G S G G G G	
TCCGACATCC AGTTGACCCA GTCTCCATCC TTCCTGTCTG CATCTGTAGG AGACAGAGTC	480
S D I Q L T Q S P S F L S A S V G D R V	
ACCATCACTT GCCGGGCCAG TCAGGGCATT AGCAGTTATT TAGCCTGGTA TCAGCAAAAA	540
T I T C R A S Q G I S S Y L A W Y Q Q K	
CCAGGGAAAG CCCCTAAGCT CCTGATCTAT GCTGCATCCA CTTTGCAAAG TGGGGTCCCA	600
P G K A P K L L I Y A A S T L Q S G V P	
TCAAGGTTCA GCGGCAGTGG ATCTGGGACA GATTTCATC TCACCATCAG CAGCCTGCAG	660
S R F S G S G S G T D F T L T I S S L Q	
CCTGAAGATT TTGCAACTTA TTA CTGTCAA CAGCTTAATA GTTACCCTTT CACTTTCGGC	720
P E D F A T Y Y C Q Q L N S Y P F T F G	
CCTGGGACCA AAGTGGATAT CAAAGCGGCC GCAGGTGC	758
P G T K V D I K	

FIG. 58

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GCCCAGCCGG CCATGGCCGA GGTGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTAAAGCCT 60
E V Q L V E S G G G L V K P

GGGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG 120
G G S L R L S C A A S G F T F S N A W M

AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA 180
S W V R Q A P G K G L E W V G R I K S K

ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA 240
T D G G T T D Y A A P V K G R F T I S R

GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC 300
D D S K N T L Y L Q M N S L K T E D T A

GTGTATTACT GTACCACATA CGGCGACGTT TACGACCGCG ATTACGATGG GCGCTGGGGT 360
V Y Y C T T Y G D V Y D R D Y D G R W G

CAAGGAACTC TGGTCACCGT CTCCTCAGGT GGAGGCGGTT CAGGCGGAGG TGGCTCTGGC 420
Q G T L V T V S S G G G G S G G G G S G

GGTGGCGGAT CCGACATCCA GATGACCCAG TCTCCATCCT CCCTGTCTGC ATCTGTAGGA 480
G G G S D I Q M T Q S P S S L S A S V G

GACAGAGTCA CCATCACTTG CCGGGCGAGT CAGGGCATTG GCAATTATTT AGCCTGGTAT 540
D R V T I T C R A S Q G I S N Y L A W Y

CAGCAGAAAC CAGGGAAAGT TCCTAAGCTC CTGATCTATG CTGCATCCAC TTTGCAATCA 600
Q Q K P G K V P K L L I Y A A S T L Q S

GGGGTCCCAT CTCGGTTCAG TGGCAGTGA TCTGGGACAG ATTTCACTCT CACCATCAGC 660
G V P S R F S G S G S G T D F T L T I S

AGCCTGCAGC CTGAAGATGT TGCAACTTAT TACTGTCAAA AGTATAACAG TGCCCTTTTC 720
S L Q P E D V A T Y Y C Q K Y N S A P F

ACTTTCGGCC CTGGGACCAA AGTGGATATC AAAACGGCCG C 761
T F G P G T K V D I K

FIG. 57

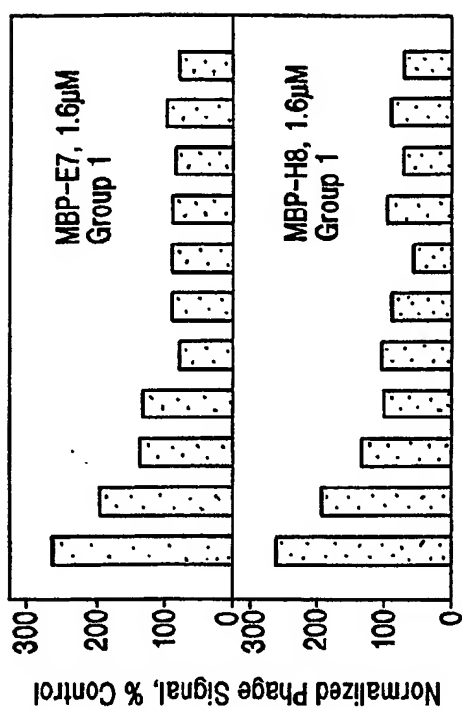


FIG. 56B

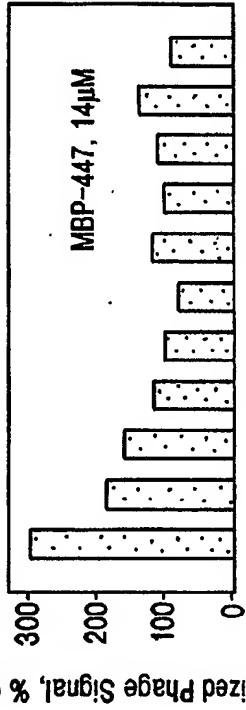


FIG. 56C

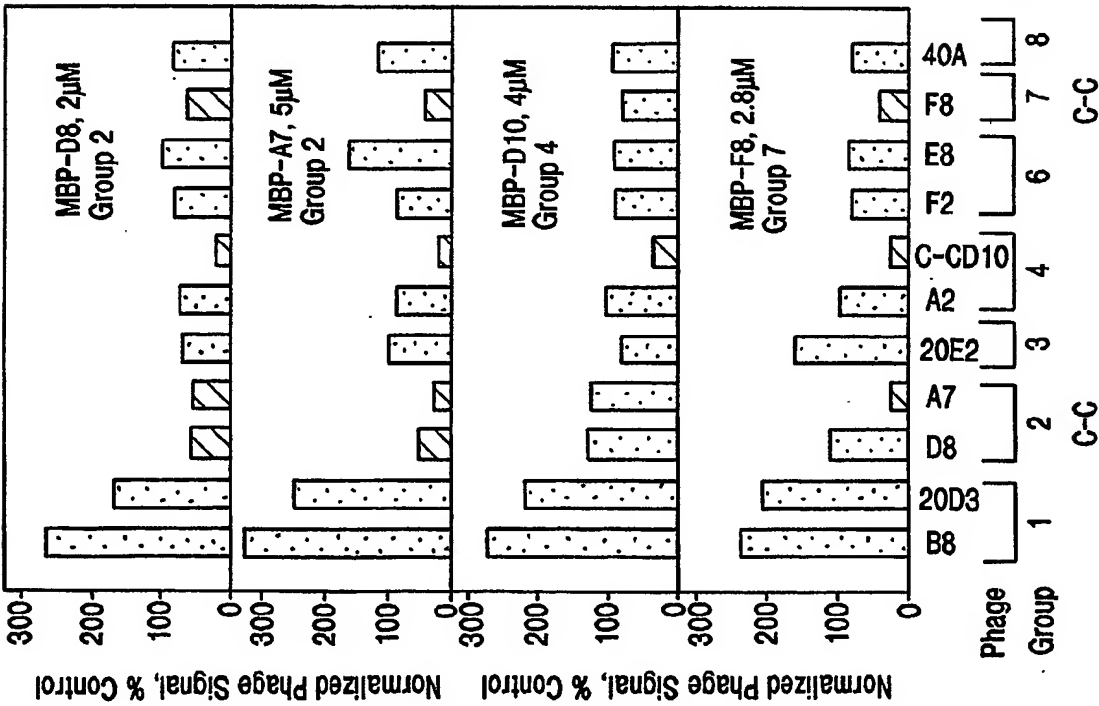


FIG. 56A

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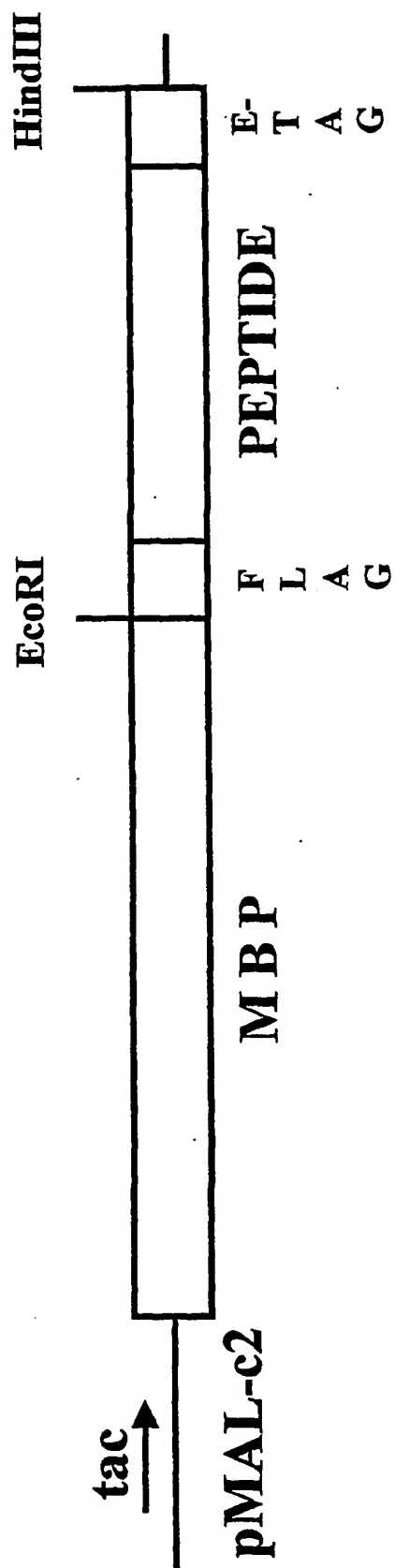


FIG. 55

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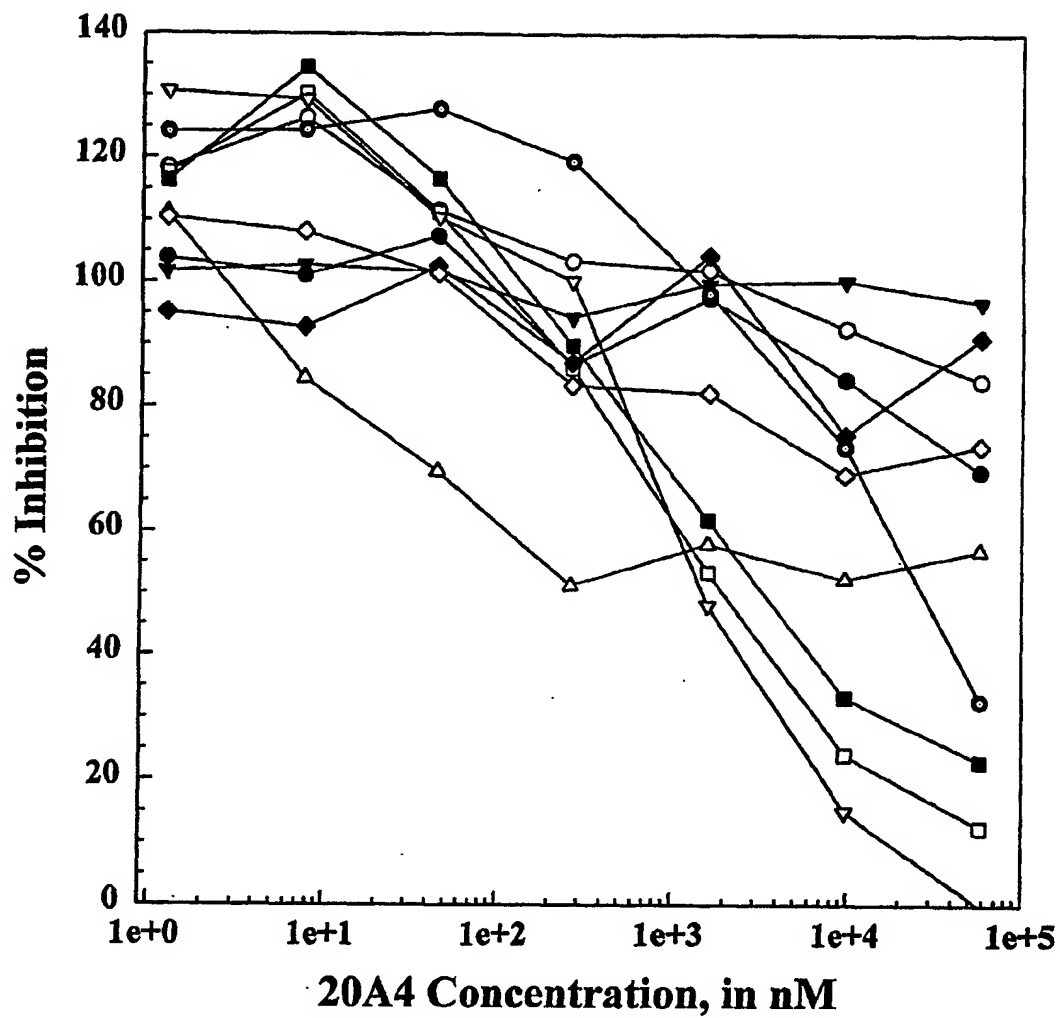


FIG. 54

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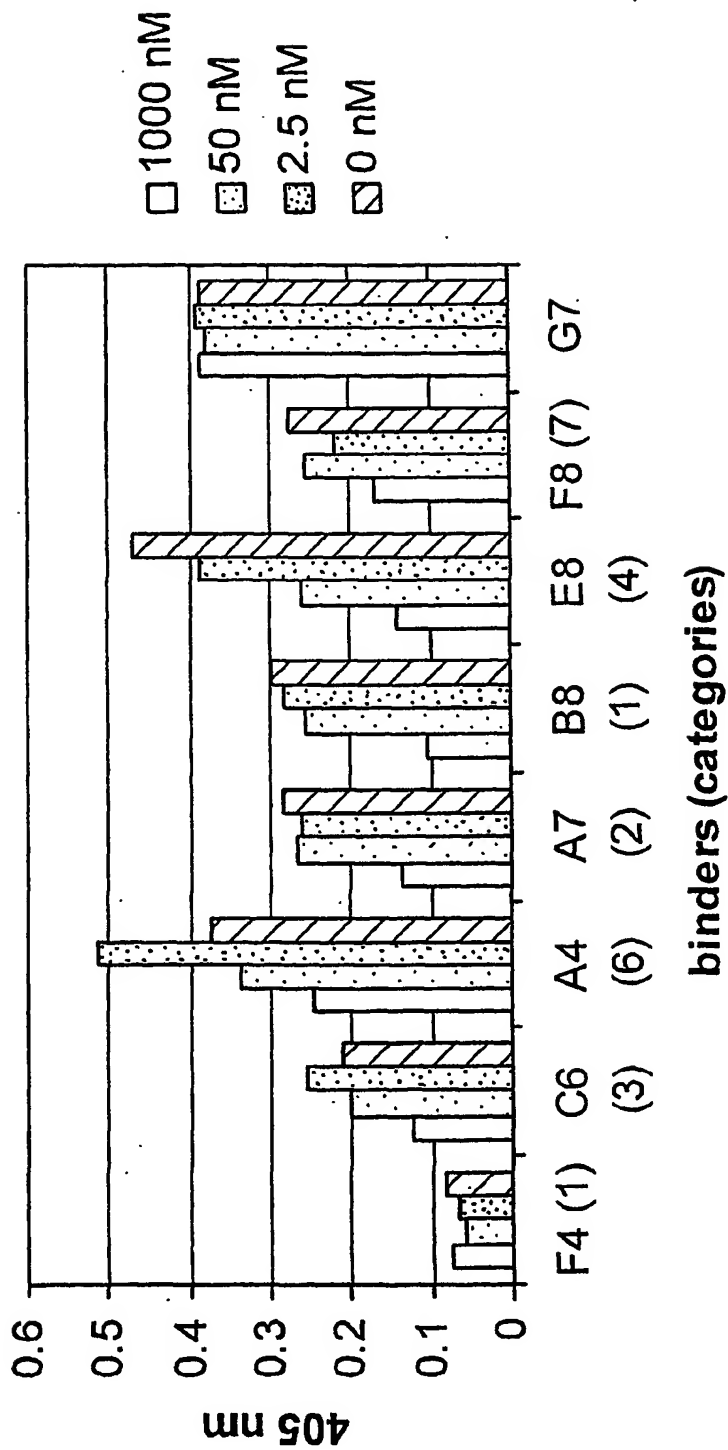


FIG. 53

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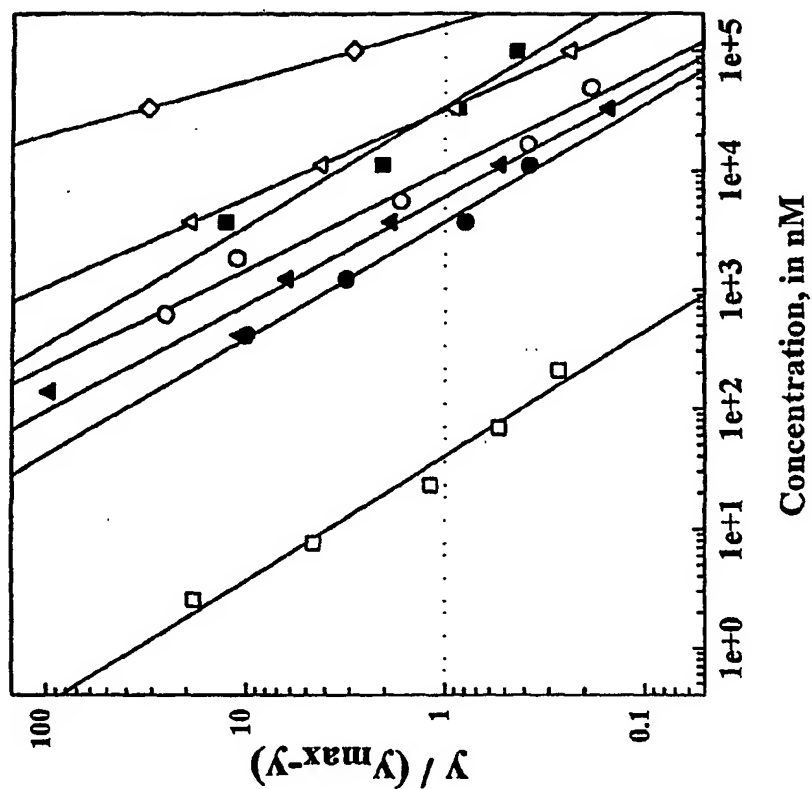


FIG. 52D

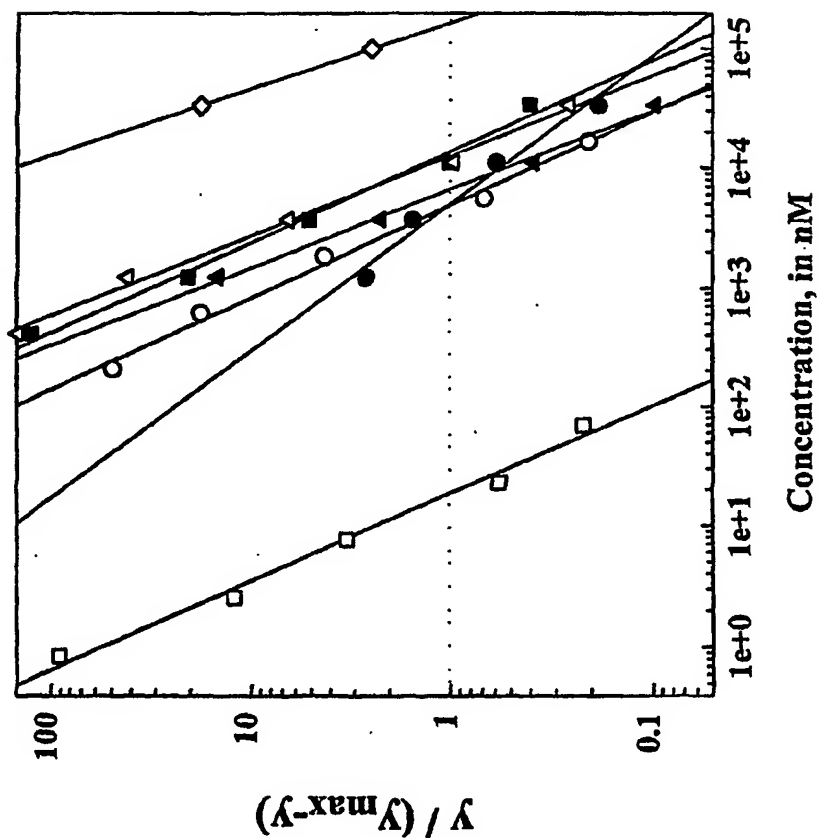
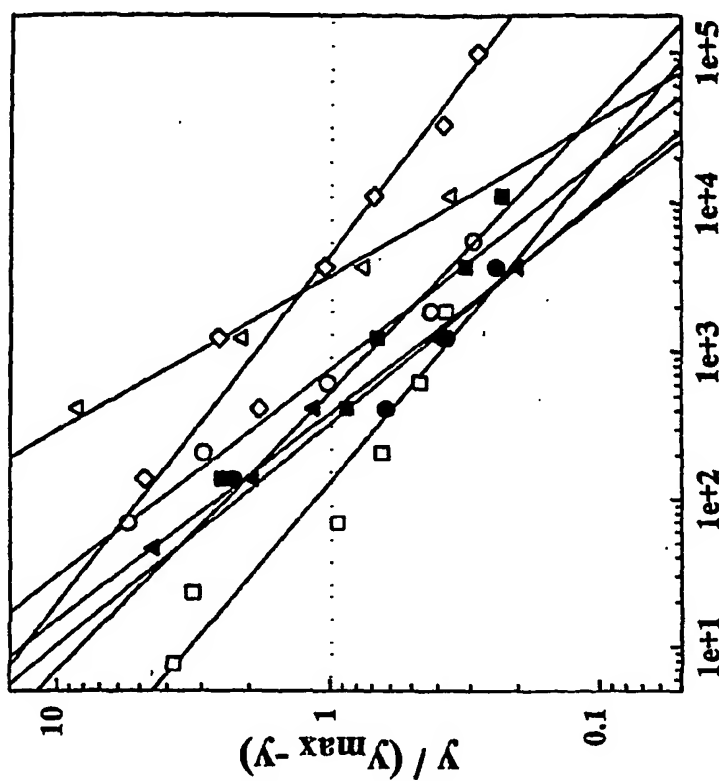


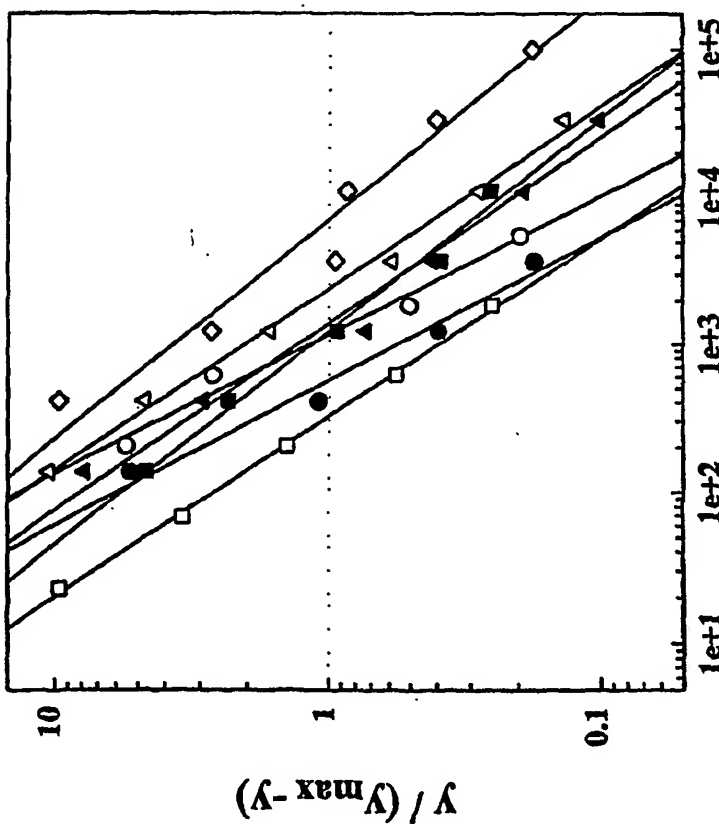
FIG. 52C

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Concentration, in nM

FIG. 52B



Concentration, in nM

FIG. 52A

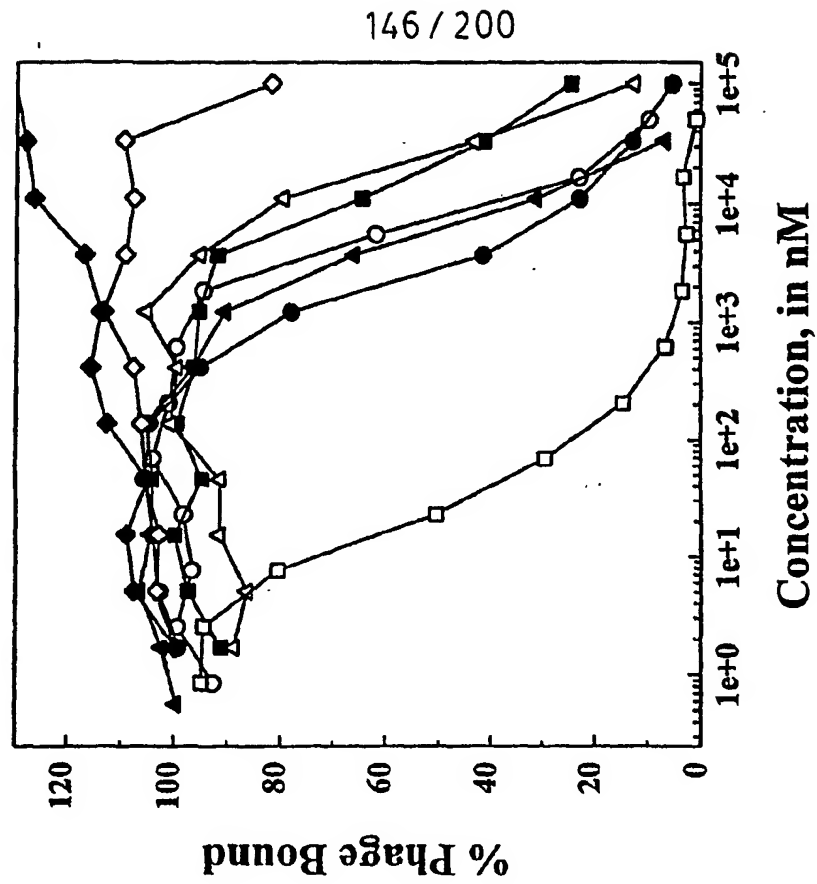


FIG. 51D

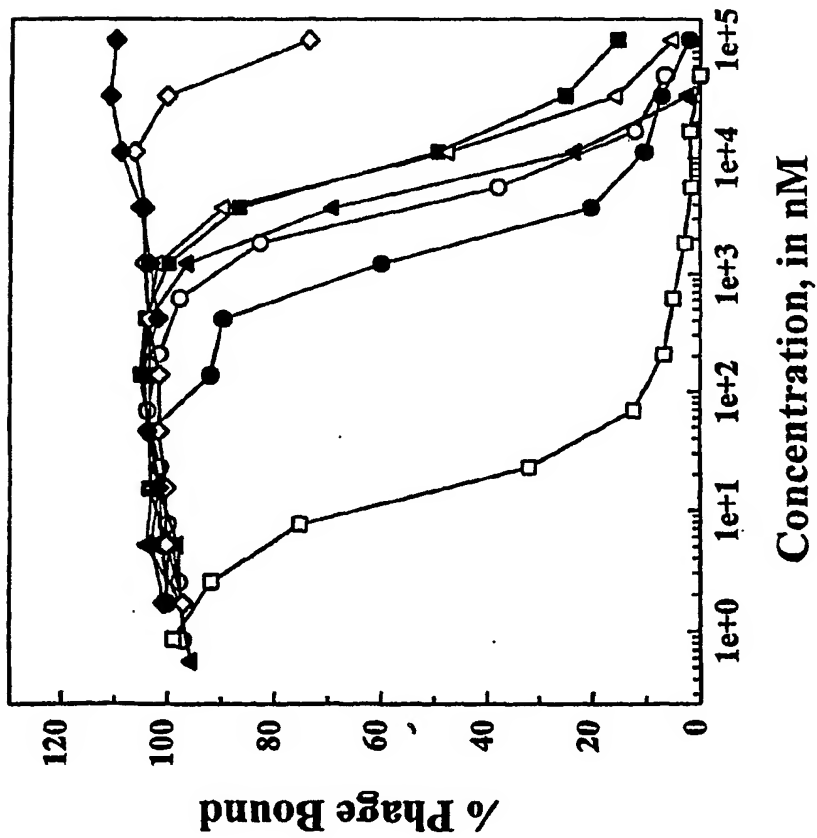
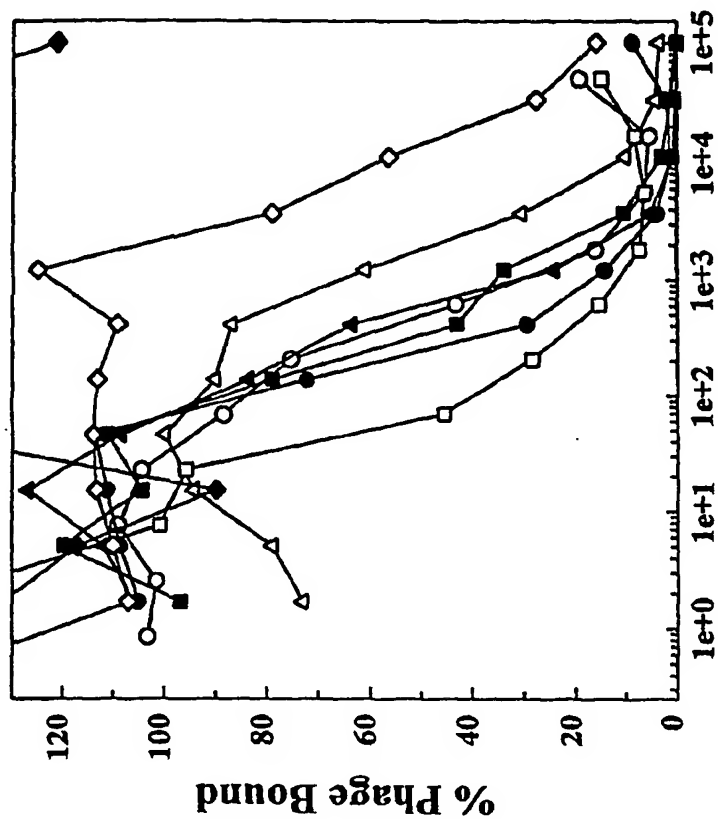


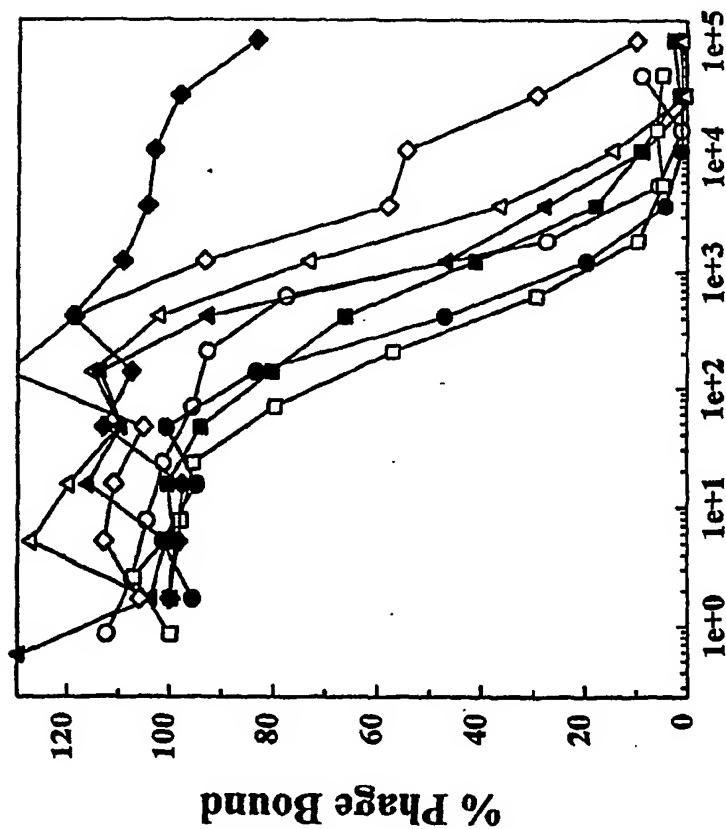
FIG. 51C

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Concentration, in nM

FIG. 51B



Concentration, in nM

FIG. 51A

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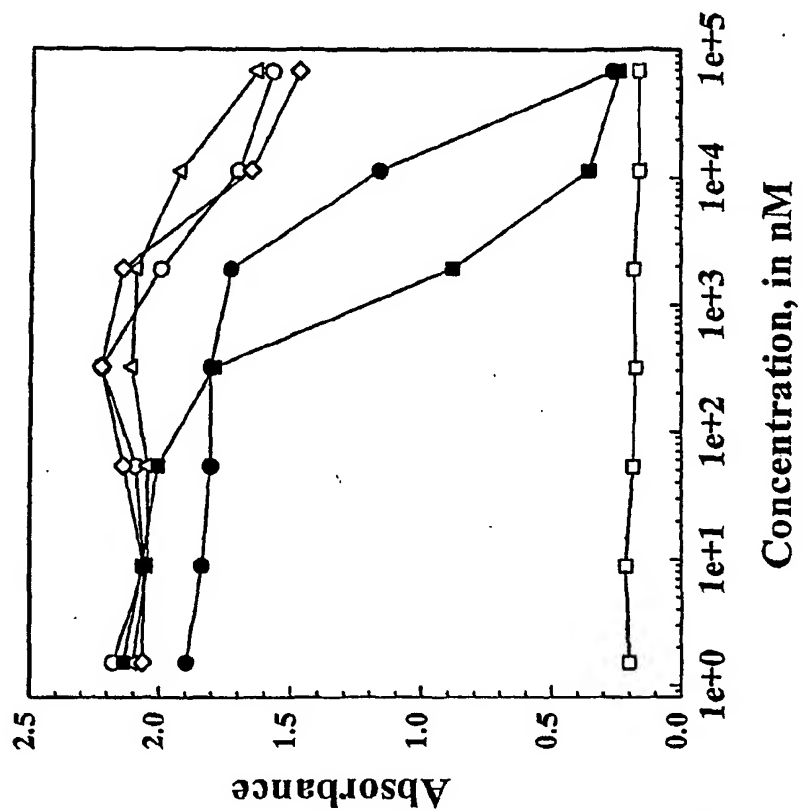


FIG. 50D

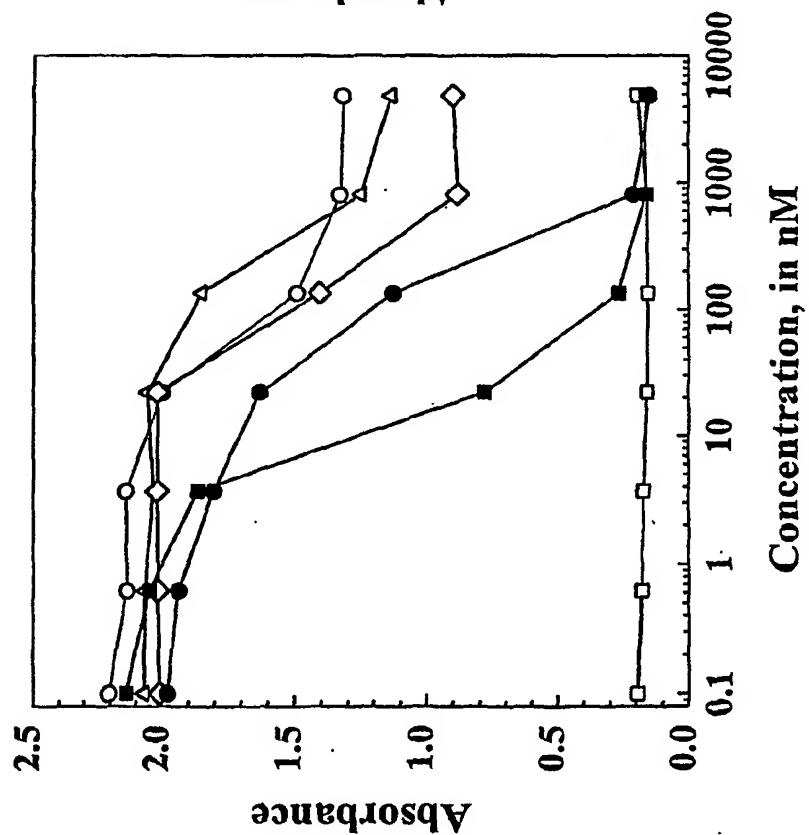


FIG. 50C

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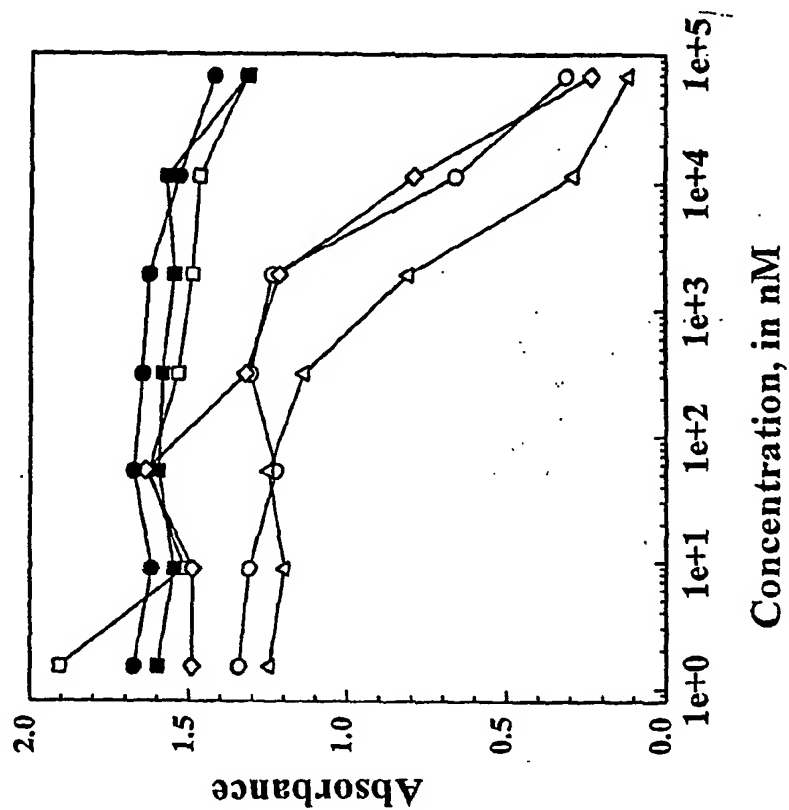


FIG. 50B

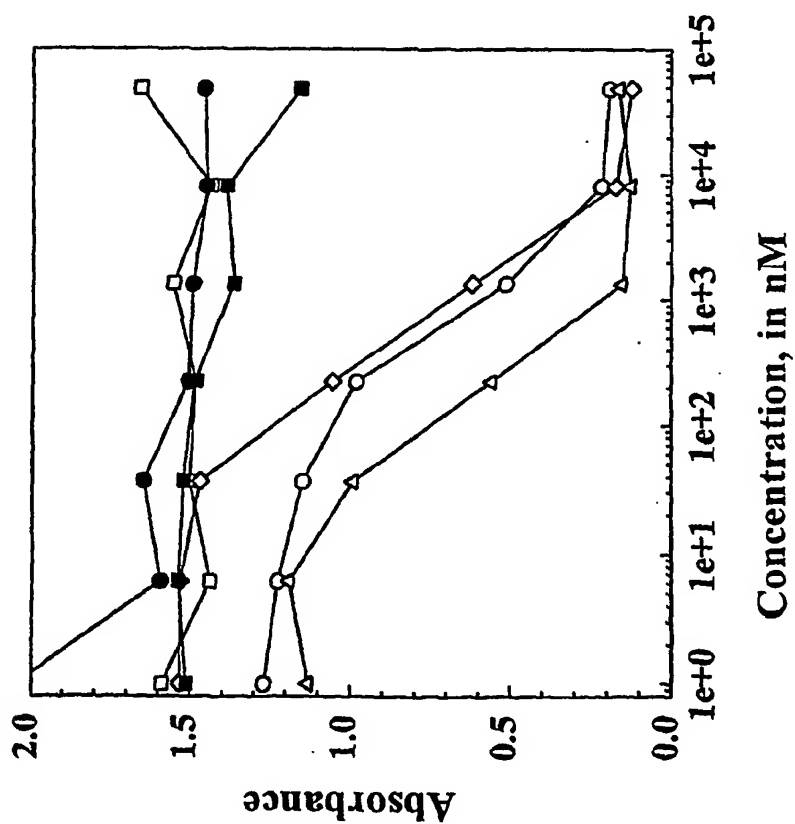


FIG. 50A

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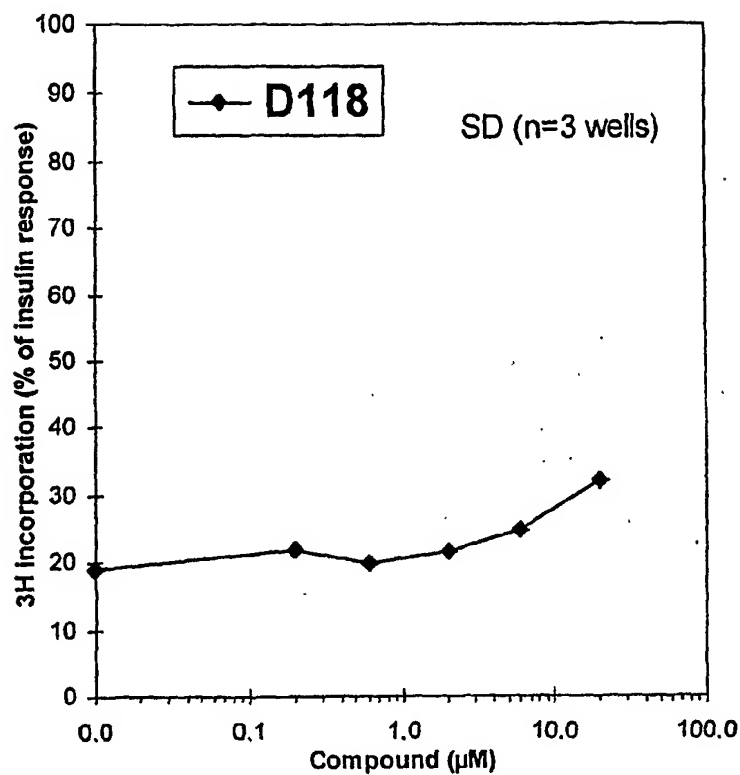


FIG. 49C

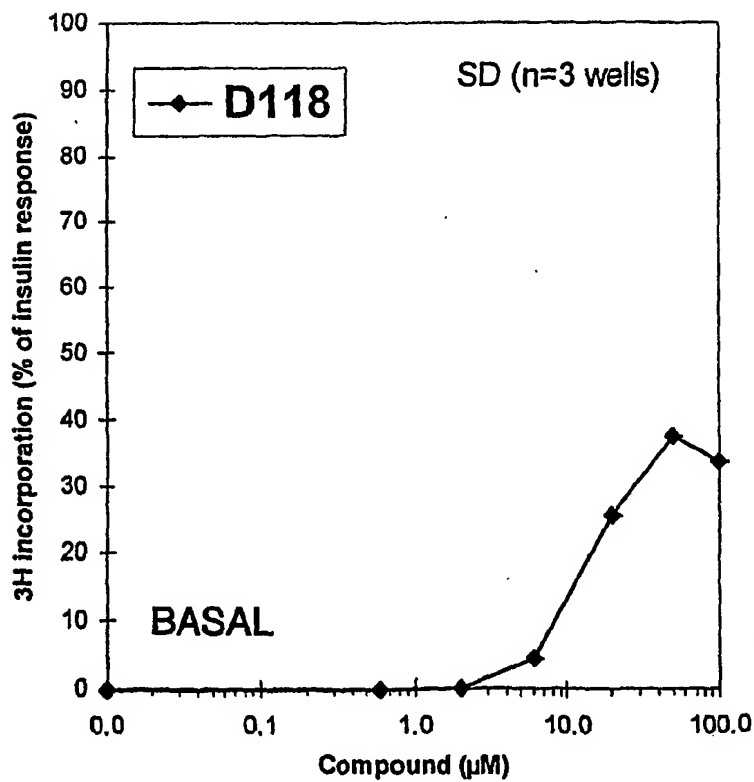


FIG. 49D

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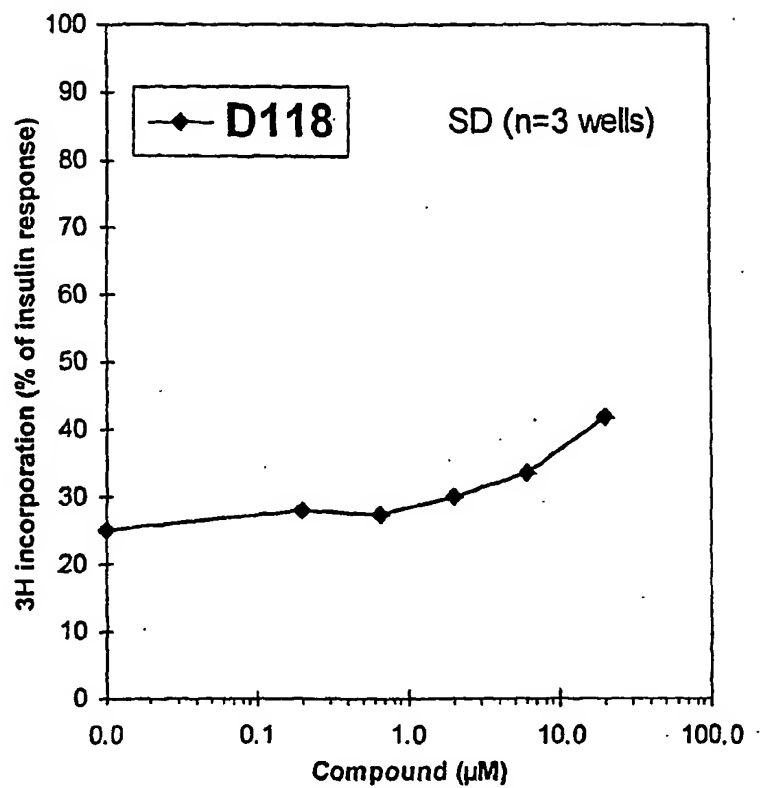


FIG. 49A

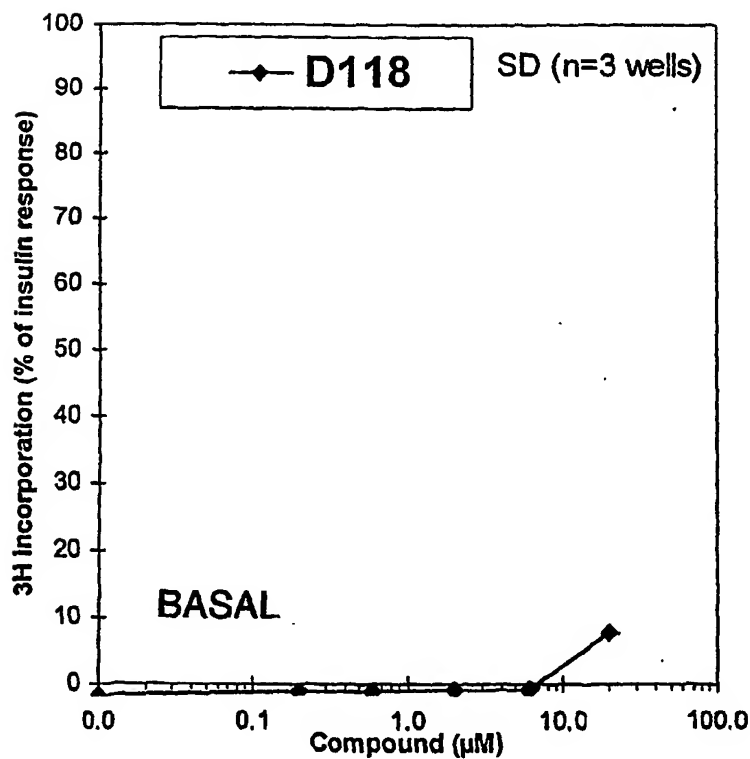


FIG. 49B

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	Found	Target	
		IR	IGF
D10	3	++	0
A2	1	++	+
F2	1	++	++
E8	1	++	?
A4	1	0	+++

Group 4 and 6: Miscellaneous Motif 10

D10 LGPLLRWGSEVCGVWPDICE
 A2 GRVALWGPVWPRWEMSRPV
 F2 SMFVAGSDRWPGYGVLADWL
 E8 VRGFQGGTVWPGYEWLRNAA
 A4 WPGYLFFEEALQDWRGSTED

	Found	Target	
		IR	IGF
B6	1	0	++
F8	4	+++	+
40D6	1	0	0

Group 7: Formula 4 Motif

B6 ACSSEFFVKGPEGFLQCLGSI
 F8 HLCVLEELFWGASLEFGYCSCG
 40D6 PERGRGLRTAMQLMRRPRDWHFFHSLFWGAPPPPLSG

Group 8: Non-Aligning
 Miscellaneous
 Sequences

FIG. 48-2

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		Target	
Found	IR	IR	IGF
13	+++	+++	0
3	+++	+++	?

		Target	
Found	IR	IR	IGF
1	+	+	++++
1	+	+	++++
1	+	+	++++
1	+	+	++++
1	+	+	+++
1	+	+	+++
1	+	+	0

		Target	
Found	IR	IR	IGF
1	++	++	++
1	+	+	+

Group 2: Formula 6 Motif

20A4* EIEAEWGRVRCLVYGRCVGG
D8 WLDQEWAWVQCEVYGRGCP

Group 3: Formula 2 Motif

20E2 DYKDFYDAIDQLVRGSARAGTRD
20C11 DYKDDRAFTYNGLRDLVGAVYGAWD
20A12 DYKDRLEFYCGIQALGANLGYSGCV
C6 DYKDFYSALWGLCGVTGCG
A6 RGQSDAFYSGLNALIGLSDG
40H4 RYFFFGGFGYGNLDVLRWLRLPYVASPRWGHWRPGGSLGKQPT

Group 5: Miscellaneous Motif 10

D9-2 PFGEGRWWGIPRMWYRNS
H4 WWWGGRNRWWLERWGLGGER

FIG. 48-1

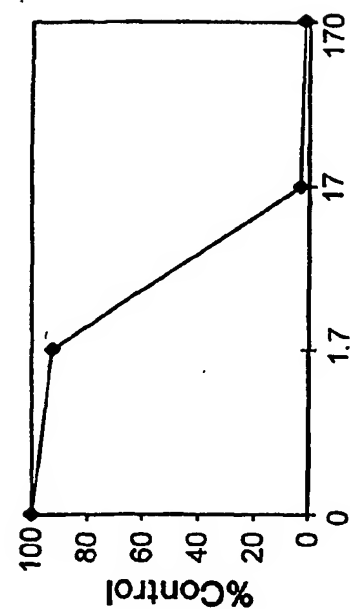
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Gr up 1 (Formula 1 Motif)	Found	Target	
		IR	IGF
20D3*	18	+	+++
20F1	16	+	+++
G3	8	+	+++
20H1	5	+	+++
20D1*	4	+	+++
D2	3	+	+++
B8	2	++	+++
C4	2	+	+++
A8	2	+	+++
E7	1	++	+++
C8	1	+	+++
H8	1	+	+++
E2	1	+	+++
B12	1	+	+++
D10-2	1	+	++
G8	1	+	++
H3	1	+	++
3G11	1	+	++
F4	1	+	++
E7-2	1	+	+
40G11	3	+	+++
40B2	2	+	++
40B12	1	+	++

IGGQGHQDGNFYDWEALAA
VFWNCRSQQLDFYEWFEQAA
RGGTFYEWFEESALRKHGAG
RVAGAISAPGLVSNKQDGLFYSWFRE
VLQARHGCDSVSDCFYEWFA
DPERMQSDVGFYEWFRRAAVG
WSALLSVMDTGFYAWFDDAV
DIGSDGHGRRWDSFYRWFEEM
IGGSFVEFYGFWENDQV
GHSWALVRHVDRRLFYEWFDL
LPAGGAQGFVAVRGFYEWFEES
RDKPTDQEEQNWSFYEWFRH
SRDQTNFTENSAGFYGWFER
GAFYRWFHEALVGSERVPDV
RIGGGWARSEGFYEWFEVREL
RMFYEWFEWSQMGAGPTEGSA
HEAFYDWFESALVDGGYELMG
FYGWFEFSRQLSLTPRDDWGLP
GVGTLTMSSDAFYTWFEV
LGTSAGQGVGHRAFYQWFQS
<---ETLESHYVVPQ-----AALDRLFYSWFS
IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFFID
RMGLQALAHYRKSA-----GPIFLSSGSVIKGSEGDPFYAWFRLQ

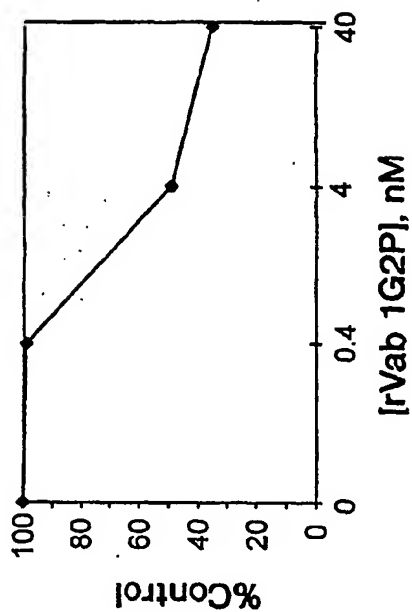
FIG. 47

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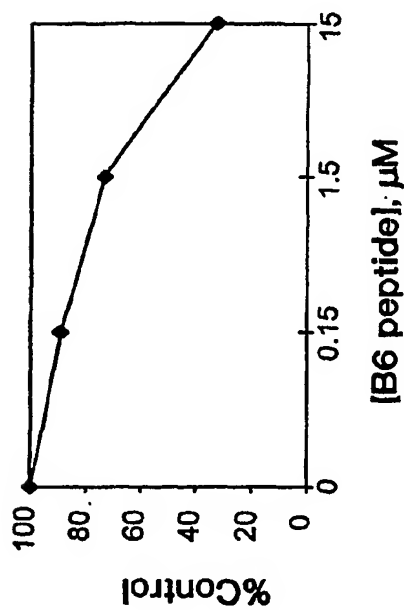
[rVab 43G7], nM

FIG. 46B



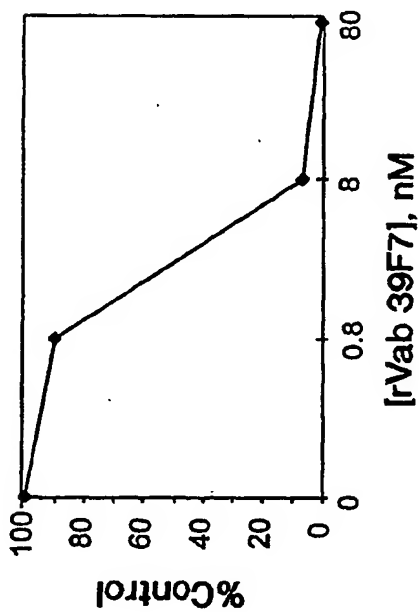
[rVab 1G2P], nM

FIG. 46D



[B6 peptide], μM

FIG. 46A



[rVab 39F7], nM

FIG. 46C

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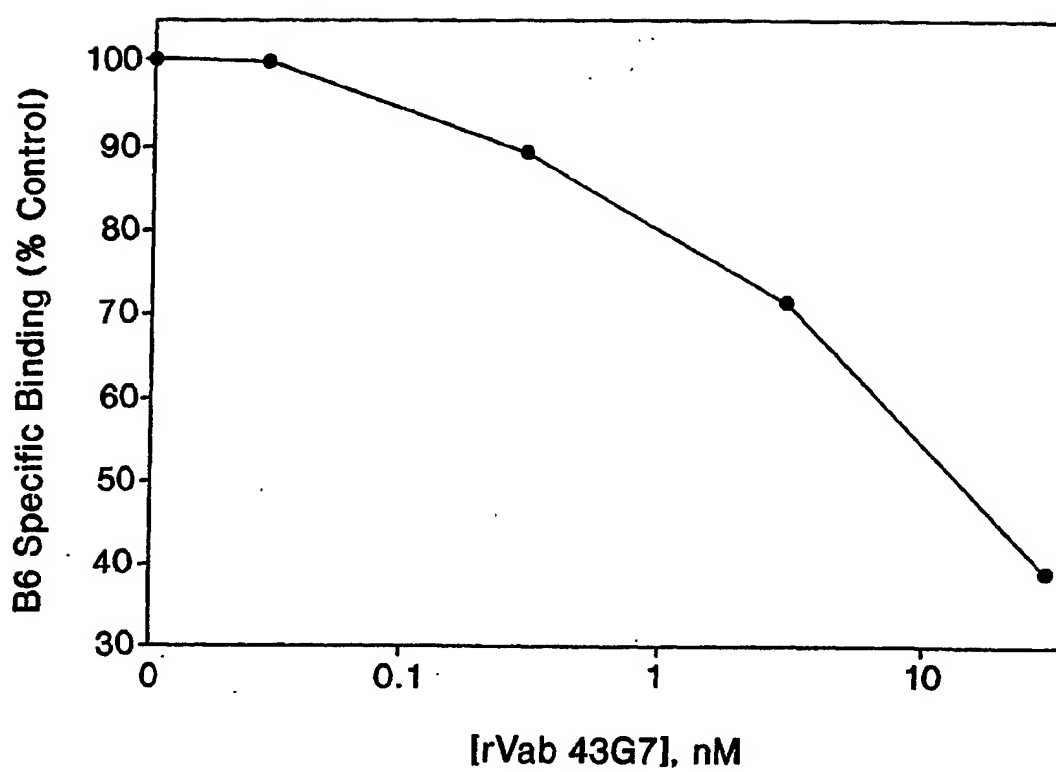


FIG. 45

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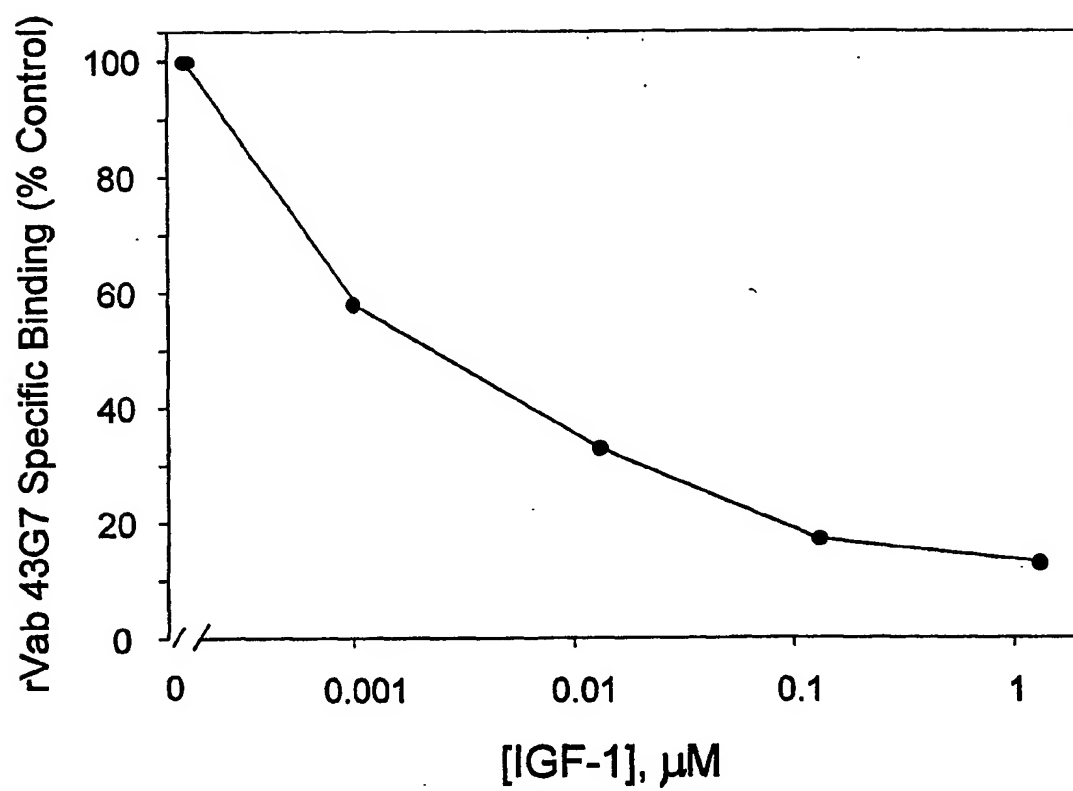


FIG. 44

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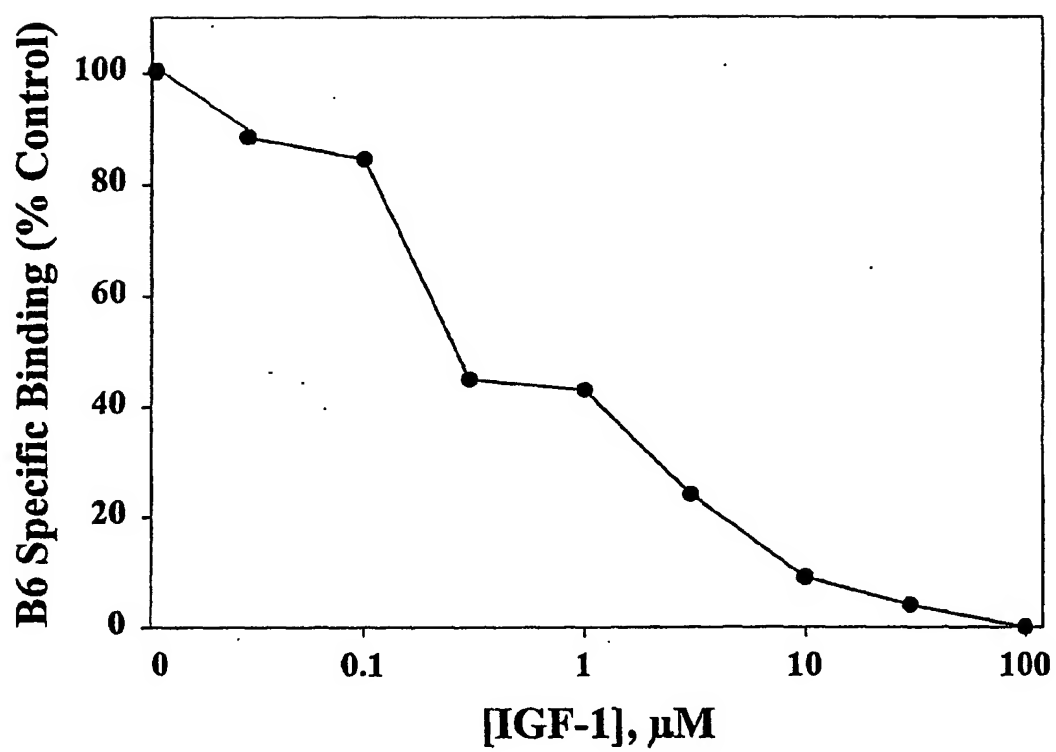


FIG. 43

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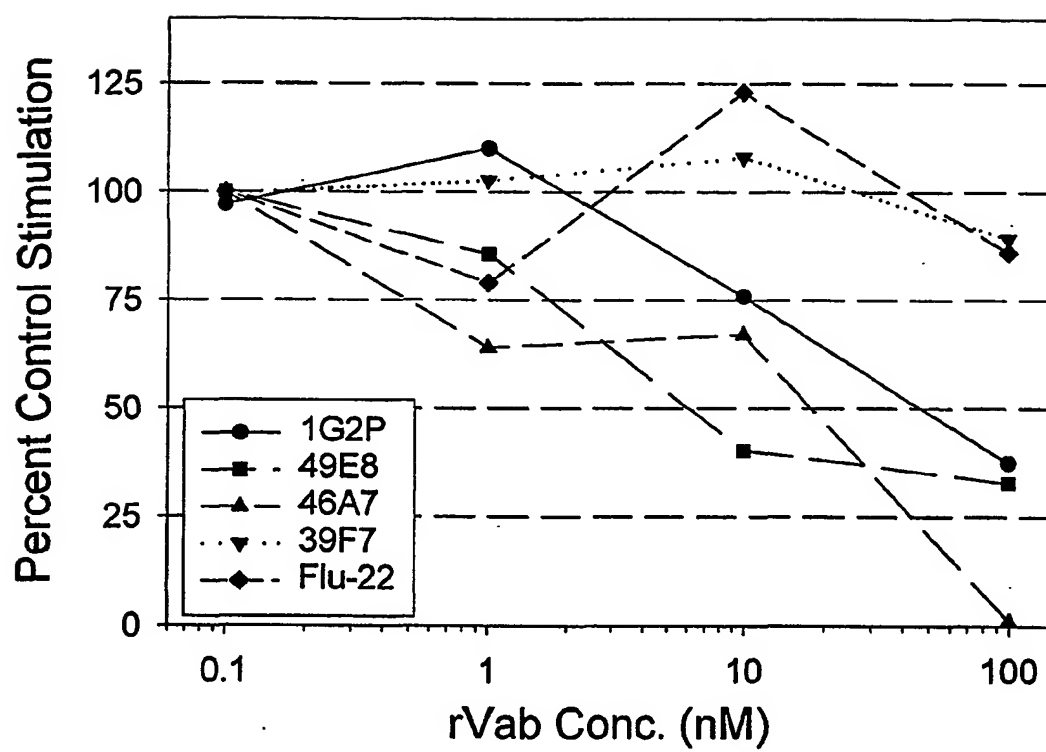


FIG. 42

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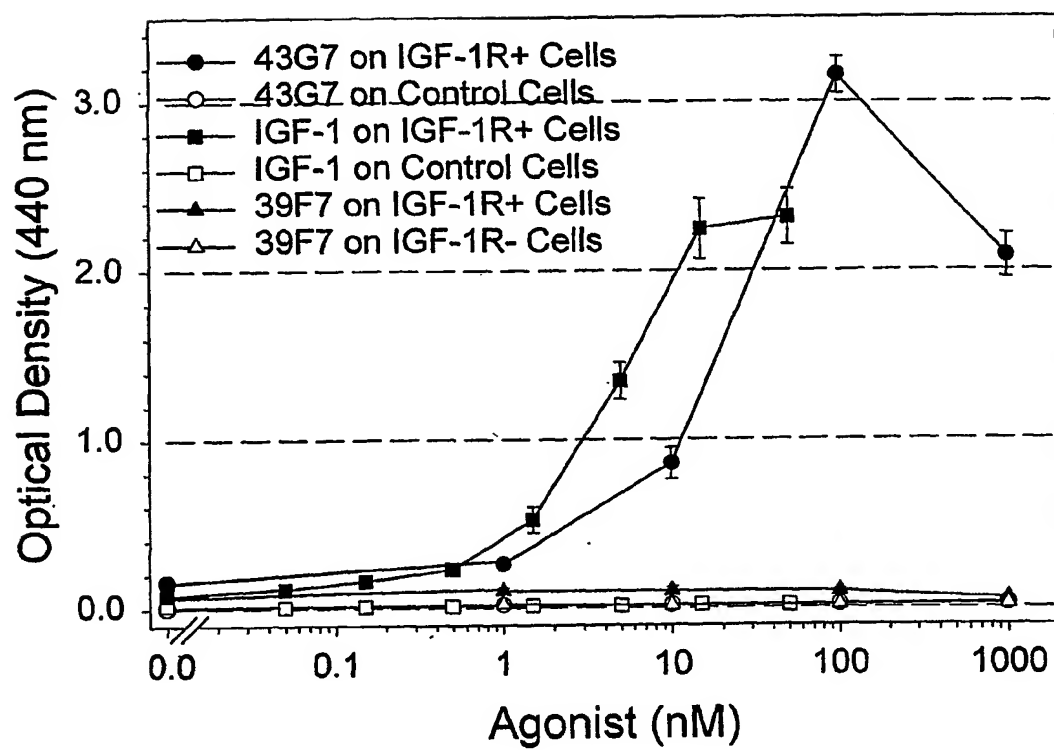


FIG. 41

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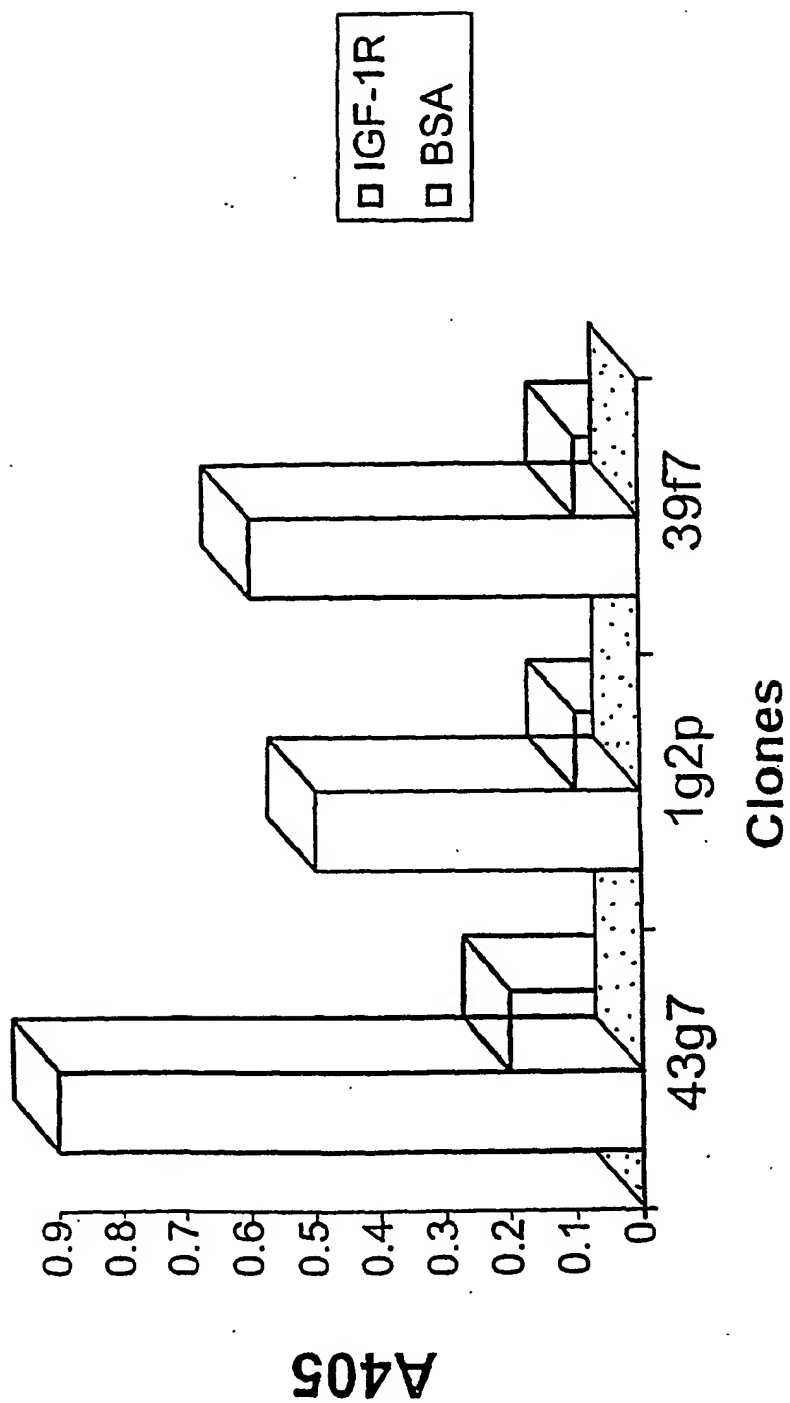


FIG. 40

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GGCCAGCCGG CCATGGCCCA GATGCAGCTG GTGCAGTCTG GGGGAGGCTT GGTACAGCCT 60
Q M Q L V Q S G G G L V Q P

GGGGGGTCCC TGAGACTCTC CTGTGCAGGC TCTGGATTCA CCTTCAGTAG CTATGCTATG 120
G G S L R L S C A G S G F T F S S Y A M

CACTGGGTTC GCCAGGCTCC AGGAAAAGGT CTGGAGTGGG TATCAGCTAT TGGTACTGGT 180
H W V R Q A P G K G L E W V S A I G T G

GGTGGCACAT ACTATGCAGA CTCCGTGAAG GGCCGATTCA CCATCTCCAG AGACAATGCC 240
G G T Y Y A D S V K G R F T I S R D N A

AAGAACTCCT TGTATCTTCA AATGAACAGC CTGAGAGCCG AGGACATGGC TGTGTATTAC 300
K N S L Y L Q M N S L R A E D M A V Y Y

TGTGCAAGAG AGGGCGAGCT CGGGGTGACC TCCTTCTGGG GTCAAGGAAC TCTGGTCACC 360
C A R E G E L G V T S F W G Q G T L V T

GTCTCCTCAG GTGGAGGCGG TTCAGGCGGA GGTGGCTCTG GCGGTGGCGG ATCCGACATC 420
V S S G G G G S G G G G S G G G G S D I

CAGATGACCC AGTCTCCATC CTCCCTGTCT GCATCTGTAG GAGACAGAGT CACCATCACT 480
Q M T Q S P S S L S A S V G D R V T I T

TGCCGGGCGA GTCAGGGCAT TAGCAATTAT TTAGCCTGGT ATCAGCAGAA ACCAGGGAAA 540
C R A S Q G I S N Y L A W Y Q Q K P G K

GTTCCCTAAGC TCCTGATCTA TGCTGCATCC ACTTTGCAAT CAGGGGTCCC ATCTCGGTTC 600
V P K L L I Y A A S T L Q S G V P S R F

AGTGGCAGTG GATCTGGGAC AGATTTCACT CTCACCATCA GCAGCCTGCA GCCTGAAGAT 660
S G S G S G T D F T L T I S S L Q P E D

GTTGCAACTT ATTACTGTCA AAAGTATAAC AGTGCCCTT GGACGTTCGG CCAAGGGACC 720
V A T Y Y C Q K Y N S A P W T F G Q G T

AAGGTGGAAA TCAAAGCGGC CGC 743
K V E I K

FIG. 39

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GCCCAGCCGG	CCATGGCCCA	GATGCAGCTG	GTGCAGTCTG	GGGGAGGCTT	GGTACAGCCT	60
	Q	M	Q	L	V	Q
		S	G	G	G	L
					V	Q
					P	
GGGGGGTCCC	TGAGACTCTC	CTGTGCAGGC	TCTGGATTCA	CCTTCAGTAG	CTATGCTATG	120
G	G	S	L	R	L	S
		C	A	G	S	G
			F	T	F	S
					S	Y
					A	M
CACTGGGTTC	GCCAGGCTCC	AGGAAAAGGT	CTGGAGTGGG	TATCAGCTAT	TGGTACTGGT	180
H	W	V	R	Q	A	P
		G	K	G	L	E
			W	V	S	A
					I	G
					T	G
GGTGGCACAT	ACTATGCAGA	CTCCGTGAAG	GGCCGATTCA	CCATCTCCAG	AGACAATGCC	240
G	G	T	Y	Y	A	D
		S	V	K	G	R
			F	T	I	S
					R	D
					N	A
AAGAACTCCT	TGTATCTTCA	AATGAACAGC	CTGAGAGCCG	AGGACATGGC	TGTGTATTAC	300
K	N	S	L	Y	L	Q
		M	N	S	L	R
			A	E	D	M
					A	V
					Y	Y
TGTGCAAGAT	GGGGGCACGT	CGGCTTGTGG	GTTGCGGACG	TCTATTGGGG	TCAAGGAACT	360
C	A	R	W	G	H	V
		G	L	W	V	A
			D	V	Y	W
					G	Q
					G	T
CTGGTCACCG	TCTCCTCAGG	TGGAGGCGGT	TCAGGCGGAG	GTGGCTCTGG	CGGTGGCGGA	420
L	V	T	V	S	S	G
		G	G	G	S	G
			G	G	G	G
					S	G
					G	G
					G	G
TCCGACATCC	AGATGACCCA	GTCTCCATCC	TCCCTGTCTG	CATCTGTAGG	AGACAGAGTC	480
S	D	I	Q	M	T	Q
		S	P	S	S	L
			S	A	S	V
					G	D
					R	V
ACCATCACTT	GCCGGGCGAG	TCAGGGCATT	AGCAATTATT	TAGCCTGGTA	TCAGCAGAAA	540
T	I	T	C	R	A	S
		Q	G	I	S	N
			Y	L	A	W
					Y	Q
					Q	K
CCAGGGAAAG	TTCCTAAGCT	CCTGATCTAT	GCTGCATCCA	CTTGGCAATC	AGGGGTCCCA	600
P	G	K	V	P	K	L
		L	I	Y	A	A
			S	T	L	Q
					S	G
					V	P
TCTCGGTTCA	GTGGCAGTGG	ATCTGGGACA	GATTTCACTC	TCACCATCAG	CAGCCTGCAG	660
S	R	F	S	G	S	G
		S	G	T	D	F
			T	L	T	I
					S	S
					L	Q
CCTGAAGATG	TTGCAACTTA	TTACTGTCAA	AAGTATAACA	GTGCCCCTTA	CACTTTTGGC	720
P	E	D	V	A	T	Y
		Y	C	Q	K	Y
			N	S	A	P
					Y	T
					F	G
CAGGGGACCA	AGCTGGAGAT	CAAAGCGGCC	GC			752
Q	G	T	K	L	E	I
				K		

FIG. 38

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GCCCAGCCGG CCATGGCCCA GGTGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTACAGCCT	60
Q V Q L V E S G G G L V Q P	
GGGGGATCCC TGAGACTCTC CTGTGCAGCC TCTGGATTCA CCTTCAGTAA CAGTGACATG	120
G G S L R L S C A A S G F T F S N S D M	
AACTGGGTCC ATCAGGCTCC AGGAAAGGGG CTGGAGTGGG TATCGGGTGT TAGTTGGAAT	180
N W V H Q A P G K G L E W V S G V S W N	
GGCAGTAGGA CGCACTATGC AGACTCTGTG AAGGGCCGAT TCATCATCTC CAGAGACAAT	240
G S R T H Y A D S V K G R F I I S R D N	
TCCAGGAACA CCCTGTATCT GCAAACGAAT AGCCTGAGGG CCGAGGACAC GGCTGTGTAT	300
S R N T L Y L Q T N S L R A E D T A V Y	
TACTGTGTGA GAACCGATGG CGAGTGGTAC GGGGCCTGGG GTCAAGGAAC TCTGGTCACC	360
Y C V R T D G E W Y G A W G Q G T L V T	
GTCTCCTCAG GTGGAGGCGG TTCAGGCGGA GGTGGCTCTG GCGGTGGCGG ATCCGCCATC	420
V S S G G G G S G G G G S G G S A I	
CAGATGACCC AGTCTCCATC CTCCCTGTCT GCATCTGTAG GAGACAGAGT CACCATCACT	480
Q M T Q S P S S L S A S V G D R V T I T	
TGCCGGGCAA GTCAGGGCAT TAGAAATGAT TTAGGCTGGT ATCAGCAGAA ACCAGGGAAA	540
C R A S Q G I R N D L G W Y Q Q K P G K	
GCCCCTAAGC TCCGGATCTA TGCTGCATCC AGTTTACAAA GTGGGGTCCC ATCAAGGTTC	600
A P K L R I Y A A S S L Q S G V P S R F	
AGCGGCAGTG GATCTGGCAC AGATTTCACT CTCACCATCA GCAGCCTGCA GCCTGAAGAT	660
S G S G S G T D F T L T I S S L Q P E D	
TTTGCAACTT ATTACTGTCT ACAAGATTAC AATTACCCTC TCACTTTCGG CGGAGGGACC	720
F A T Y Y C L Q D Y N Y P L T F G G G T	
AAGGTGGAGA TCAAAGCGGC CGC	743
K V E I K	

FIG. 37

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GCCCAGCCGG CCATGGCCCA GATGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTAAGCCT Q M Q L V E S G G G L V K P	60
GGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG G G S L R L S C A A S G F T F S N A W M	120
AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA S W V R Q A P G K G L E W V G R I K S K	180
ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA T D G G T T D Y A A P V K G R F T I S R	240
GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC D D S K N T L Y L Q M N S L K T E D T A	300
GTGTATTACT GTACCACACC GGGCTGGTAT GGGGCCGAGG ATAAGTGGGG TCAAGGAACT V Y Y C T T P G W Y G A E D K W G Q G T	360
CTGGTCACCG TCTCCTCAGG TGGAGGCGGT TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA L V T V S S G G G G S G G G G S G G G G	420
TCCGACATCC AGATGACCCA GTCTCCATCC TCCCTGTCTG CATCTGTAGG AGACAGAGTC S D I Q M T Q S P S S L S A S V G D R V	480
ACCATCACTT GCCGGGCGAG TCAGGGCATT AGCAATTATT TAGCCTGGTA TCAGCAGAAA T I T C R A S Q G I S N Y L A W Y Q Q K	540
CCAGGGAAAG TTCCTAAGCT CCTGATCTAT GCTGCATCCA CTTTGCAATC AGGGGTCCCA P G K V P K L L I Y A A S T L Q S G V P	600
TCTCGTTCA GTGGCAGTGG ATCTGGGACA GATTTCCTC TCACCATCAG CAGCCTGCAG S R F S G S G S G T D F T L T I S S L Q	660
CCTGAAGATG TTGCAACTTA TTAAGTGTCAA AAGTATAACA GTGCCCCTTT CACTTTCGGC P E D V A T Y Y C Q K Y N S A P F T F G	720
CCTGGGACCA AAGTGGATAT CAAAGCGGCC GC P G T K V D I K	752

FIG. 36

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GCCCAGCCGG CCATGGCCCA GATGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTAAAGCCT 60
 Q M Q L V E S G G G L V K P

GGGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG 120
 G G S L R L S C A A S G F T F S N A W M

AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA 180
 S W V R Q A P G K G L E W V G R I K S K

ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA 240
 T D G G T T D Y A A P V K G R F T I S R

GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC 300
 D D S K N T L Y L Q M N S L K T E D T A

GTGTATTACT GTACCACATG GGGCTCCGTC GACACGGACA ACTACGCCAG GTTTTGGGGT 360
 V Y Y C T T W G S V D T D N Y A R F W G

CAAGGAACTC TGGTCACCGT CTCCTCAGGT GGAGGCGGTT CAGGCGGAGG TGGCTCTGGC 420
 Q G T L V T V S S G G G G S G G G G S G

GGTGGCGGAT CCGACATCCA GATGACCCAG TCTCCATCCT CCCTGTCTGC ATCTGTAGGA 480
 G G G S D I Q M T Q S P S S L S A S V G

GACAGAGTCA CCATCACTTG CCGGGCGAGT CAGGGCATTG GCAATTATTT AGCCTGGTAT 540
 D R V T I T C R A S Q G I S N Y L A W Y

CAGCAGAAAC CAGGGAAAGT TCCTAAGCTC CTGATCTATG CTGCATCCAC TTTGCAATCA 600
 Q Q K P G K V P K L L I Y A A S T L Q S

GGGGTCCCAT CTCGGTTCAG TGGCAGTGGG TCTGGGACAG ATTTCACTCT CACCATCAGC 660
 G V P S R F S G S G S G T D F T L T I S

AGCCTGCAGC CTGAAGATGT TGCAACTTAT TACTGTCAAA AGTATAACAG TGCCCTCTC 720
 S L Q P E D V A T Y Y C Q K Y N S A P L

ACTTTCGGCG GAGGGACCAA GGTGGAGATC AAAGCGGCCG C 761
 T F G G G T K V E I K

FIG. 35

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GGCCAGCCGG CCATGGCCCA GGTGCAGCTG GTGGAGTCTG GGGGAGGCTT GGTAAAGCCT 60
Q V Q L V E S G G G L V K P

GGGGGGTCCC TTAGACTCTC CTGTGCAGCC TCTGGATTCA CTTTCAGTAA CGCCTGGATG 120
G G S L R L S C A A S G F T F S N A W M

AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CTGGAGTGGG TTGGCCGTAT TAAAAGCAAA 180
S W V R Q A P G K G L E W V G R I K S K

ACTGATGGTG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCAGATTCAC CATCTCAAGA 240
T D G G T T D Y A A P V K G R F T I S R

GATGATTCAA AAAACACGCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAGCC 300
D D S K N T L Y L Q M N S L K T E D T A

GTGTATTACT GTACCACAGT TGC GTTGTCT GCCGACCGTG GGATGTGGGG TCAAGGAACT 360
V Y Y C T T V A L S A D R G M W G Q G T

CTGGTCACCG TCTCCTCAGG TGGAGGCGGT TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA 420
L V T V S S G G G G S G G G G S G G G

TCCGATGTTG TGATGACTCA GTCTCCACTC TCCCTGCCCC TCACCCTTGG ACAGCCGGCC 480
S D V V M T Q S P L S L P V T L G Q P A

TCCATCTCCT GCAGGTCTAG TCAAAGCCTC GTATACAGTG ATGGAAACAC CTACTTGAAT 540
S I S C R S S Q S L V Y S D G N T Y L N

TGGTTTCAGC AGAGGCCAGG CCAATCTCCA AGGCGCCTAA TTTATAAGGT TTCTAACCGG 600
W F Q Q R P G Q S P R R L I Y K V S N R

GACTCTGGGG TCCCAGACAG ATTCAGCGGC AGTGGGTCAG GCACTGATTT CACACTGAAA 660
D S G V P D R F S G S G S G T D F T L K

ATCAGCAGGG TGGAGGCTGA GGATGTTGGG GTTTATTACT GCATGCAAGG TACACACTGG 720
I S R V E A E D V G V Y Y C M Q G T H W

CCTTACACTT TTGGCCAGGG GACCAAGCTG GAGATCAAAG CGGCCCGC 767
P Y T F G Q G T K L E I K

FIG. 34

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Lambda 3 genes:	DPL16 (=v3s1)+ / +v318 / Ser. His NNN-----CAT GGNN-----G	/ JL2/3 for DPL16+v3s1+v318 / NotI / PCR primer site / ValValPheGlyGlyGlyThrLysLeuThrValLeu GTGGTATTTCGGCGGAGGACCAAGCTGACCGTCCTAGCGGCGCGCAGTGTGAGTCCAAAGATTTCG TACACCATAGCGCCCTCCCTGGTTCGACTGGCAGGATCGCCGGCGTCACTCAGGTTTCTAAGC
Lambda 3 gene:	/DPL23 (=VL3.1)/ Ser Ala NNN-----GCA GGNN-----C	for DPL23+VL3.1 GTG--- continued as for JL2/3 GTCAC---
Lambda 1 genes:	/ DPL2+DPL3 / Gln Gly NNN-----GGT GGNN-----C	for DPL2+DPL3 GTG--- continued as for JL2/3 CACAC---
Lambda 2 gene:	/ DPL11 / Gln Leu NNN-----CTC GGNN-----G	for DPL11 continued as for JL2/3 GTG--- AGCAC---

FIG. 33B

Ec RI / SfiI / VH gene / CDR H3 / JH4b gene / G4S linker
 CCGGAATTCGGCCAGCGCGCCNNN-Arg
 GGCCTTAAGCGGGTCGGCGGNNN-----N
 3' end for 40 VH genes
 CCGGAATTCGGCCAGCGCGCCNNN-----NAA
 GGCCTTAAGCGGGTCGGCGGNNN-----N
 3' end for 7 VH genes: DP31, DP33, DP40, DP47 and DP49
 CCGGAATTCGGCCAGCGCGCCNNN-----NCA
 GGCCTTAAGCGGGTCGGCGGNNN-----N
 3' end for 4 VH genes: DP2, DP3, DP5 and DP38
 All kappa genes:
 Asp Pro
 NNN-----CCT
 GGNNN-----G
 (except VK L20)
 TcPThrPheGlyGlnGlyThrLysValGluLeuLys
 TGACGTTTCGGCCAGGACCAAGTGGAAATCAAAGCGCGCAGTGTGAGTCCAAAGATTTCG
 GAACCTGCAAGCGGTCCCTGGTTCCACCTTAGTTTCGCGCGGTCAACACTCAGGTTTCTTAAGC
 TcPThrPheGlyGlnGlyThrLysLeuGluLeuLys
 TACACTTTTGGCCAGGACCAAGCTGGAGATCAA continued as for JK1
 GAAATGAAAACCGGTCCCTGGTTTCGACCTCTAGTT
 PheThrPheGlyProGlyThrLysValAspLeuLys
 TTCACCTTCGGCCCTGGGACCAAGTGGATACAA continued as for JK1
 GAAATGAAAACCGGACCTCGTTTCACCTATAGTT
 TcPThrPheGlyGlnGlyThrLysValGluLeuLys
 CTCACCTTCGGCGGAGGACCAAGTGGAGATCAA continued as for JK1
 GAGAGTGAAGCCGCTCCCTGGTTTCACCTCTAGTT

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FIG. 33A

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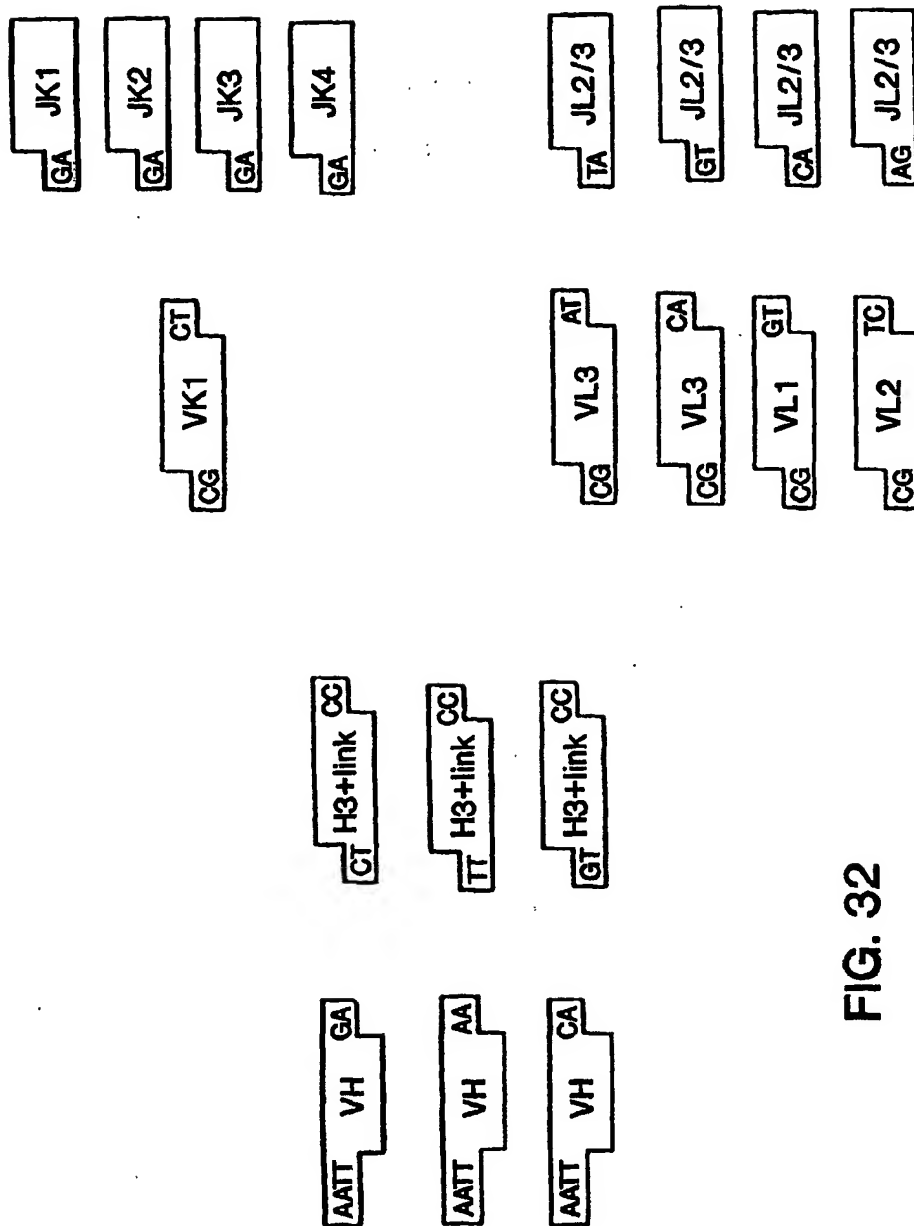


FIG. 32

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VH Gene Sequences

DP-1
DP-10
DP-12
DP-14
DP-15
DP-2
DP-21
DP-25
DP-29
DP-30
DP-31
DP-32
DP-33
DP-35
DP-38
DP-39
DP-40
DP-42
DP-44
DP-45
DP-46
DP-47
DP-5
DP-50
DP-51
DP-52
DP-53
DP-54
DP-59
DP-63
DP-66
DP-67
DP-68
DP-69
DP-7
DP-70
DP-71
DP-73
DP-74
DP-8
hv1263
VHD26

Lambda and Kappa Gene Sequences

DPK11
DPK15
DPK18
DPK2/L14+
DPK3/L11+
DPK4
DPK6
DPK8/Vd+
DPL23
HK101
L22+
L23/L23a
LFVK431
VA++

FIG. 31

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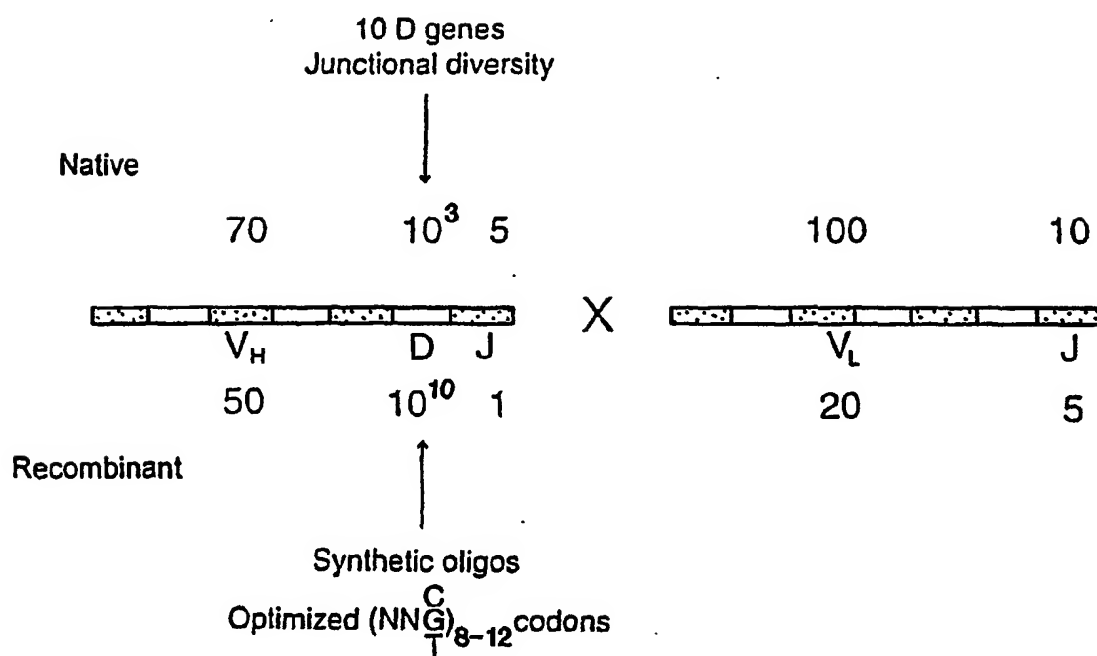
Genomic $rVab$ Library

FIG. 30

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Name	Sequence	#Found	Ratio	IGF Inh.	GHR ⁺
H5:	LCQSLGVTPGWLAWCA	-	1.2	-	2.6
2C3:	VCQRLGGTFPGWLGVCR	-	1.1	-	1.1
JBA5:	LCQSWGVRI-GWLAGLCP	19	~24.0	~45%	1.2
E2A12:	LCQSLGFTDLWLACWFE	10	~17.5	~54	1.1
E1A11:	VCQSLGITDLGLCAGWGA	1	16.4	50	1.0
E4B10:	LCQSLGLTHPGFEAWLCA	5	~11.7	~50	1.2
E4C10:	LCQNFVTDPGCFYGFWA	1	9.9	~51	0.7
E4A9:	LCQSSGLSFLGCL-GWWA	14	~8.5	~65	1.0
JBB6:	PCQRLGDTHLCWLAGWFA	6	~8.3	~65	1.1
E1F9	PCQSLGLTCSGWFEWGA	1	8.3	68	1.2
E4G7:	QWQSLGVTCPGSWAELCA	1	6.0	50	1.3
E4A11:	LMQSVGIKYPGGLAGWLA	1	5.8	67	1.4
E1B9:	LCQSLGVTYWEGLAWLCA	3	5.5	60	1.1
E4A12:	VCQGLGVECPGWFAWGA	3	~5.3	~55	1.2
E4F11:	LCQGWGIRI-GWLVGRCM	1	2.7	58	1.1
E1D3:	LCQSLGVTPGWLAWGCA	1	2.0*	-	1.0

FIG. 29

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A6S-4-H5	DYKD	HHSPGNEHGYNFYDWFVLqVA	AAA	24	19	123
A6S-4-E4	DYKD	ERSAAGFREGNFYDWFVAqVN	AAA	32	27	120
A6S-4-F5	DYKD	GSQHSGREPHNFYDWFVAqVG	AAA	28	24	120
A6S-4-D4	DYKD	IARMRETFqPNFYDWFVDQLA	AAA	21	18	118
A6S-4-C6	DYKD	RLDRSSTSGVNFYDWFVAqVG	AAA	28	25	116
A6S-4-D3	DYKD	GLRSEQGNRLNFYDWFVAqIA	AAA	23	20	116
A6S-4-F2	DYKD	SVIqTRqDETNFYDWFV?AMS	AAA	26	23	115
A6S-4-A5	DYKD	VEVqRHIRKDNFYDWFVKQID	AAA	22	19	115
A6S-4-C3	DYKD	VTMLDKGAqDNFYDWFVREVA	AAA	24	21	114
A6S-4-F3	DYKD	HNSSSPMRTGNFYDWFVqELR	AAA	30	26	113
A6S-4-B4	DYKD	ERSPRPALASNFYDWFVQqVV	AAA	21	19	113
A6S-4-B6	DYKD	SDARqAGLQENFYDWFVSQVR	AAA	26	23	113
A6S-4-B1	DYKD	RHERGKEGPGNFYDWFVSqVV	AAA	21	19	112
A6S-4-G4	DYKD	SALSGPVqPINFYDWFVTGM	AAA	30	26	112
A6S-4-A6	DYKD	HVEHMAVGDDGNFYDWFVVqLR	AAA	23	21	111
A6S-4-F4	DYKD	VGHSGVPPYPNFYDWFVMqVS	AAA	24	22	110
A6S-4-D6	DYKD	LGAAETWDGINFYDWFVKqVS	AAA	24	22	110
A6S-4-E6	DYKD	RSSGGLLSqGNFYDWFVSQLE	AAA	26	24	109
A6S-4-A3	DYKD	LAINDLVTHKNFYDWFVDqLR	AAA	20	18	109
A6S-4-E3	DYKD	RGMTGMVGRGNFYDWFVGqLR	AAA	23	21	109
A6S-4-A2	DYKD	IGGQGqHQDGNFYDWFVEALA	AAA	22	20	107
A6S-4-B2	DYKD	qSVDLSRPDSNFYDWFVEVLS	AAA	22	21	105
A6S-4-H2	DYKD	VTFTSAVFHENFYDWFVRqVS	AAA	20	19	104
A6S-4-D1	DYKD	SNPSRqDASVNFYDWFVREVA	AAA	22	22	103
A6S-4-H1	DYKD	IVAGARHSEVNFYDWFVIqVR	AAA	18	18	102
A6S-4-E2	DYKD	?DGQSVSSKGNFYDWFVQqMT	AAA	25	25	101
A6S-4-G1	DYKD	AELVGAGVRGNFYDWFVDqLV	AAA	16	16	101
A6S-4-G2	DHKD	SAGHHMPRESNFYDWFVDqVV	AAA	24	25	99
A6S-4-A1	DYKD	DSSRLWLGERNFYDWFVAqIS	AAA	12	17	68

FIG. 28-2

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Clone:	Binding Ratios:			
	Target	E-Tag	% Max	
A6S-1-C5	DYKD RIHNQTERGGNFYDWFVHqLV AAA	7	27	26
A6S-1-G3	DYKD VATVHVGGGMNFYDWFVAqVG AAA	5	19	26
A6S-1-A2	DYKD KDPVTVSQGRNFYDWFVVqIQ AAA	5	20	25
A6S-1-D5	DYKD RVGSGMEDLGNFYDWFVRQAq AAA	5	25	20
A6S-1-H4	DYKD HKSWTTMSPLNFYDWFVAqVE AAA	3	18	17
A6S-2-F2	DYKD LAMSVASRPANFYDWFVAqIV AAA	30	35	86
A6S-2-D2	DYKD RAERGSMRDSNFYDWFVqQLP AAA	30	36	83
A6S-2-E3	DYKD VqEGLSGMEGNFYDWFVDQLF AAA	28	36	78
A6S-2-H2	DYKD RGqRESDSGTNFYDWFVGAI R AAA	28	40	70
A6S-2-A3	DYKD SRAPYGSTAGNFYDWFVqAVS AAA	25	37	68
A6S-2-H1	DYKD RVGIqVDPHTNFYDWFVIQLT AAA	27	42	64
A6S-2-F1	DYKD VGqVGRYVRSNFYDWFVQqAM AAA	8	30	27
A6S-2-G1	DYKD RPqLVESGSKNFYDWFVqVVR AAA	8	30	27
A6S-2-B2	DYKD EMYGDTSERVNFYDWFVSALq AAA	5	30	17
A6S-2-A1	DYKD LSSRGRVTMRNFYDWFVAqVV AAA	3	31	10
A6S-3-E10	DYKD RVREKLPRPENFYDWFVNqIH AAA	22	23	96
A6S-3-G2	DYKD TWMWEERKqDNFYDWFVGQLK AAA	20	21	95
A6S-3-E5	DYKD RYRGERHDGRNFYDWFVEqVN AAA	19	21	90
A6S-3-H2	DYKD qGAEGRLSEGNFYDWFVQAVS AAA	19	21	90
A6S-3-H9	DYKD YSIEVqDWNENFYDWFVSQLG AAA	20	23	87
A6S-3-G3	DYKD PRLHMGSDMGDFYDWFVVqIA AAA	18	21	86
A6S-3-F8	DYKD GRGqGLKRPDNFYDWFVAAAK AAA	20	25	80
A6S-3-G10	DYKD GAVGLAEAGPNFYDWFVSqVq AAA	19	24	79
A6S-3-H1	DYQD PASNKNSLAENFYDWFVqQTR AAA	23	30	77
A6S-3-E6	DYKD DARDHGVVMSNFYDWFVAqVS AAA	5	20	25
A6S-3-D9	DYKD SLQGADFqQGNFYDWFVSELA AAA	4	17	24
A6S-3-E3	DYKD RPSLP EVRPGNFYDWFVqSVR AAA	4	19	21
A6S-3-H8	DYKD NPTSVqQYGVNFYDWFVNVLS AAA	4	20	20
A6S-3-G4	DYKD CADPGACSSLNFYDWFVqMRG AAA	4	21	19
A6S-3-B10	DYKD YDqDPPYWGLNFYDWFVREVA AAA	3	16	19
A6S-3-C1	DYKD RPYIGGGGTRNFYDWFVAqMI AAA	3	17	18
A6S-4-G5	DYKD QEVTRTRDDKNFYDWFVSqIF AAA	26	18	144
A6S-4-D2	DYKD PPYRSSRLGENFYDWFVMqVR AAA	28	19	143
A6S-4-F6	DYKD LKGSSqPLSVNFYDWFVQQIK AAA	24	17	142
A6S-4-H4	DYKD PRMVEKPSEDNFYDWFVTqLS AAA	28	20	141
A6S-4-C1	DYKD CWARPCGDAANFYDWFVqQAS AAA	22	16	141
A6S-4-G3	DYKD GAQAIREIHNNFYDWFVAqVT AAA	29	21	139
A6S-4-H3	DYKD GRGDQRHETT NFYDWFVRELq AAA	28	20	137
A6S-4-H6	DYKD GSIAQLIMRANFYDWFVEqTN AAA	24	18	130
A6S-4-G6	DYKD RLMGGIAEPqNFYDWFVREVA AAA	25	20	126

FIG. 28-1

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GACTACAAAGACGACGATGACAAGTACCGTGGTATGCTGGTTCTGGGTCGTATCTCTGACG
 D Y K D D D D K Y R G M L V L G R I S D
 GTGCTGGTAAAGTTGCTTCTGAACCGCCGGCTCGTATCGGTCAGAAAGTTTTCGCTGTTAA
 G A G K V A S E P P A R I G Q K V F A V N
CTTCTACGACTGGTTTCGTTGCGGCCGCA 96 nt
 F Y D W F V A A A

FIG. 25A

CTACAAAGACGACGATGACAAGTACCGTGGTATGCTGGTTCTGGGTCGTATCTCTGACG
GTGCTGGTAAAGTTGCTTCTGAACCGCCGGCTCGTATCGGTCAGAAAGTTTTCGCTGTT
 AACTTCTACGACTGGTTTCGTTGCGGCCGCA GTGA 154 nt

FIG. 25B

GACTACAAAGACNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKAACTTCTACGACTGGTTTCG
 D Y K D X X X X X X X X X X X N F Y D W F

TTNNKNNKNNKNNK 21 aa
 V X X X X

FIG. 26A

CTACAAAGACNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKAACTTCTACGACTGGTTTCGTTNNK
 NNKNNKNNKGCGGCCGCA GTGTGA

FIG. 26B

H5	NH ₂ -D-Y-K-D-L-C-Q-S-L-G-V-T-Y-P-G-W-L-A-G-W-C-A-K-K(Biotin)-COOH
H5 Control	NH ₂ -D-Y-K-D-W-C-L-T-L-Q-P-L-V-W-A-S-G-G-G-Y-C-A-K-K(Biotin)-COOH
2C3-60	NH ₂ -D-Y-K-D-V-C-Q-R-L-G-G-T-F-P-G-W-L-V-G-V-C-R-K-K(Biotin)-COOH
H5-447	NH ₂ -D-Y-K-D-L-C-Q-R-L-G-V-G-W-P-G-W-L-S-G-W-C-A-K-K(Biotin)-COOH
H5-432	NH ₂ -D-Y-K-D-L-C-Q-S-L-G-V-T-W-P-G-W-L-A-G-W-C-A-K-K(Biotin)-COOH

FIG. 27

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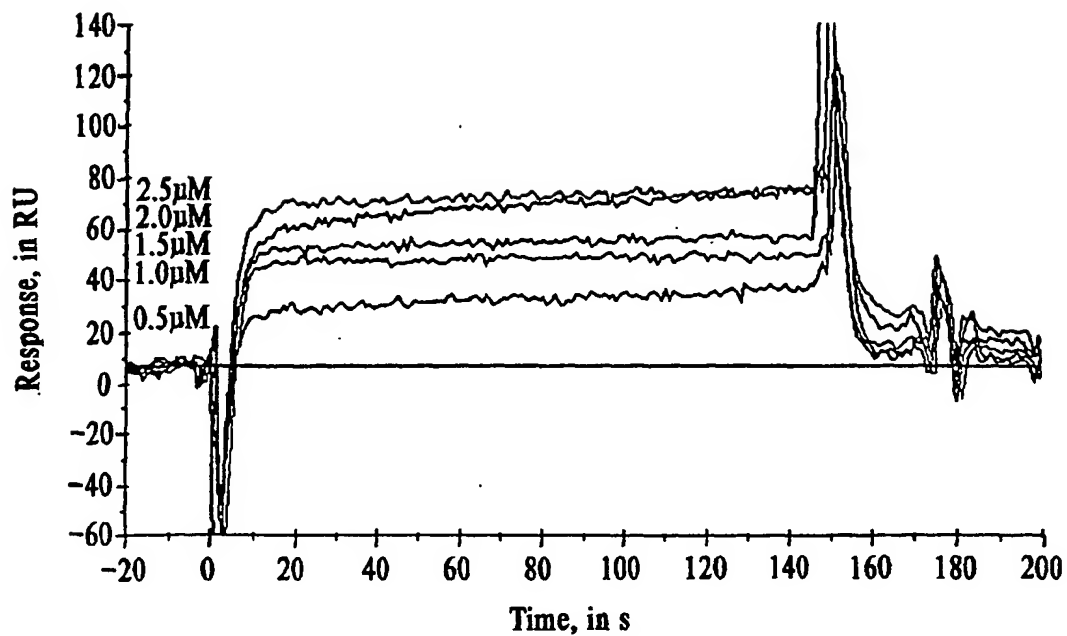


FIG. 24A

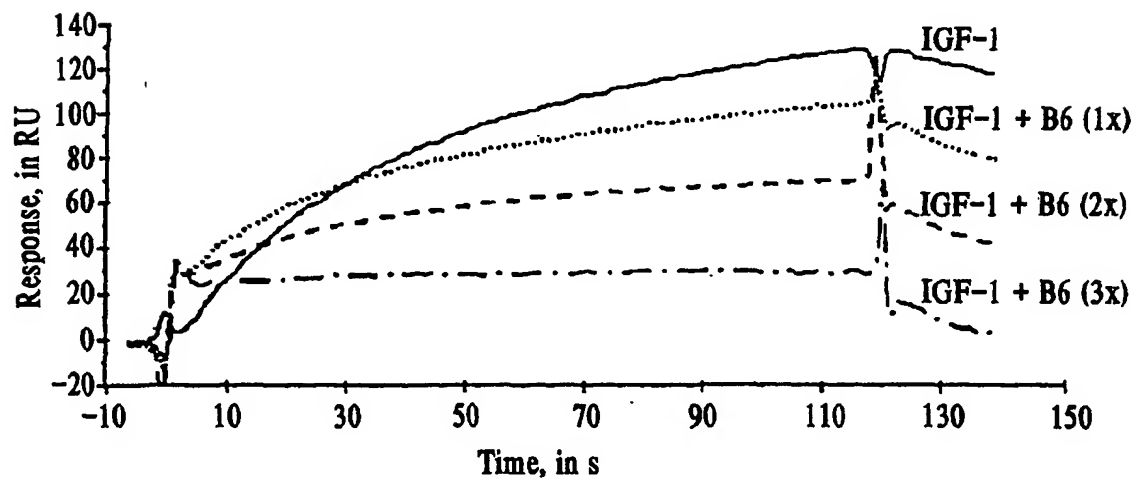


FIG. 24B

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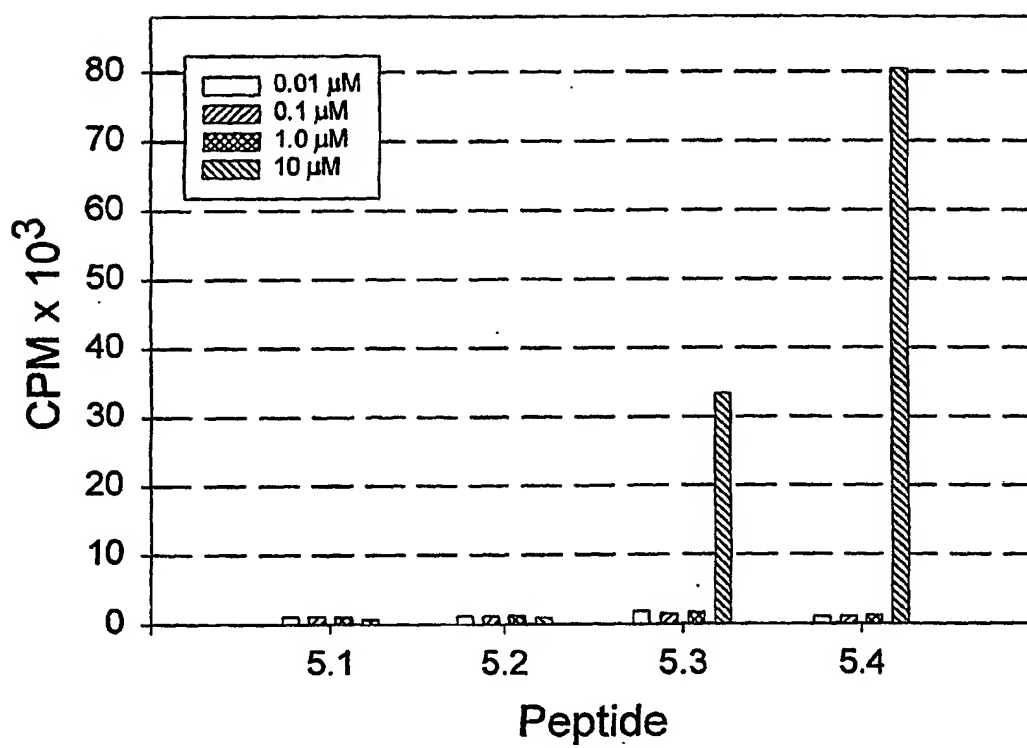


FIG. 23

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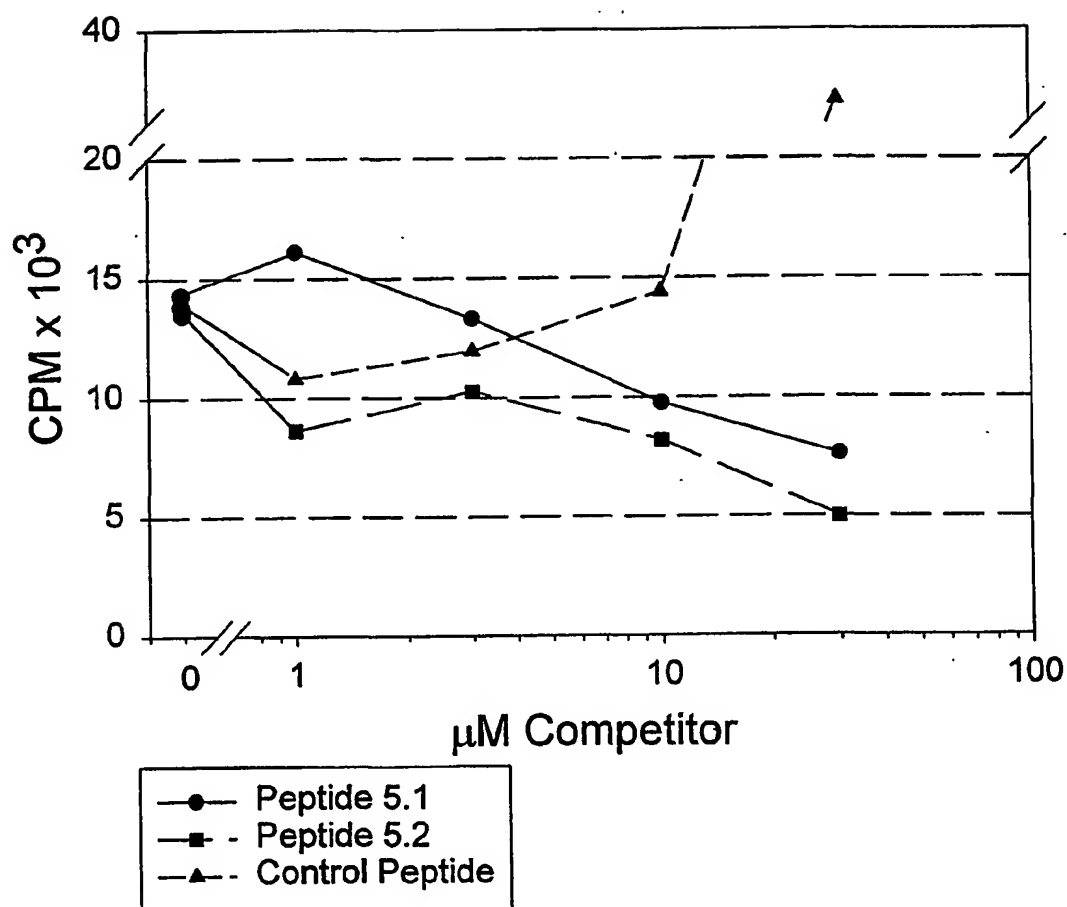


FIG. 22

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Clone B6 Peptide 5.1 (18 aa)	AETPAQVGWNRLNSVWPGEHWNTVDFFYHKLSELLRESGA NTVDFFYHKLSELLREKK (biotin)
Clone F6 Peptide 5.2 (17 aa)	MLLGVLRFQIILLWPFPKDCVQMKDIFYSLLASL QMKDIFYSLLASLAACK (biotin)
Clone D5 Peptide 5.3 (14 aa)	PLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWFEM ADKNFYDWFMAACK (biotin)
Clone A6 Peptide 5.4 (12 aa)	YRGMLVLGRISDGAGKVASEPPARIGQKVFVNFYDWFV SAKNFYDWFVKK (biotin)

FIG. 21

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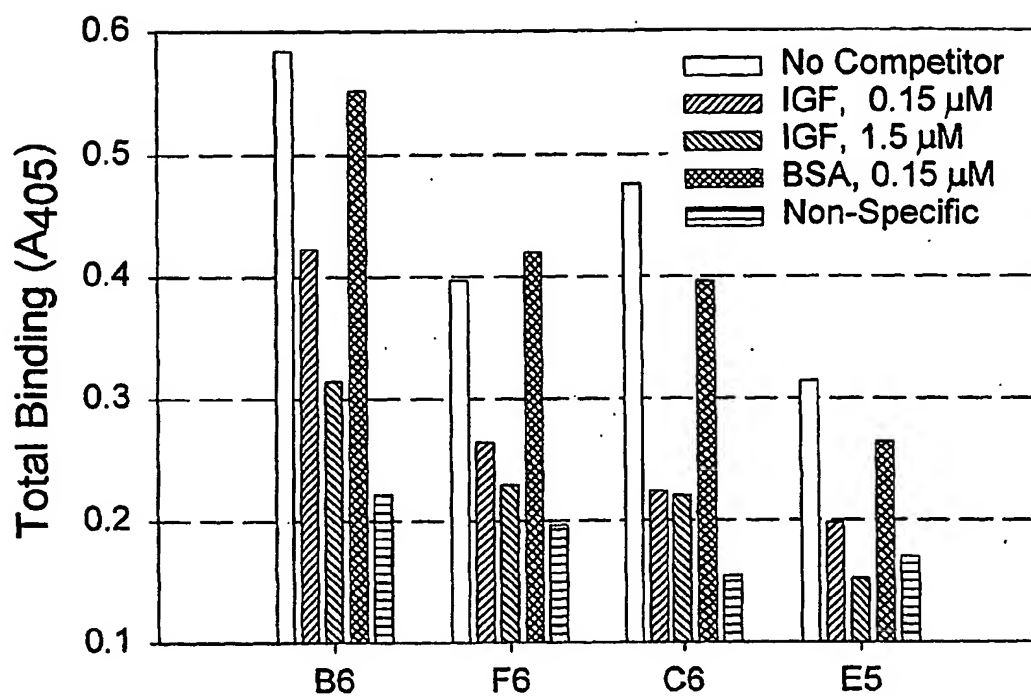
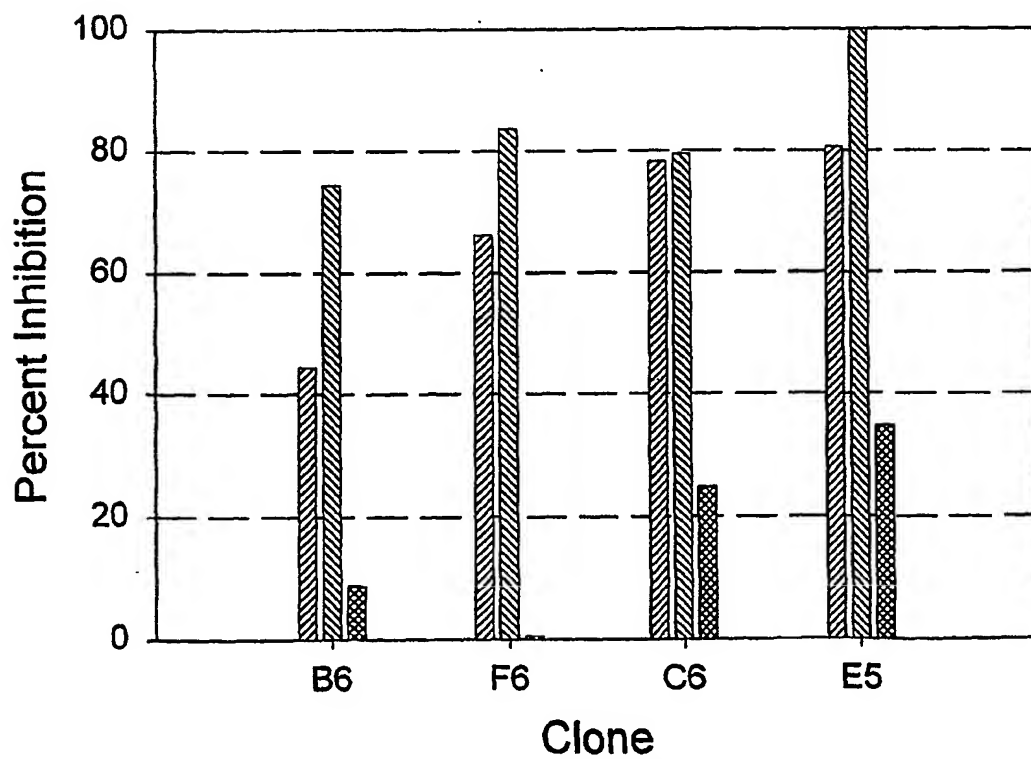


FIG. 20A

Clone
FIG. 20B

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IGF-5

GACTACAAAG ACTCGTGGTT GAATTTTCGG TATGTTGCTG GGCGTGCTTA GGTTTCAGAT TCTTCTGTGG
 D Y K D S W L N F R Y V A G R A Q V S D S S V A
 T T K T R G . I F G H L L G V L R F Q I L L W
 L Q R L V V E F S V C C W A C L G F R F F C G

CCGTTTCCTA AGGATTGTGT TCAGATGAAA GATATTTTTT ATTCGTTGTT GGCTAGTTTG GCGGCCGCA 139
 V S . G L C S D E R Y F L F V V G Q F G G R
 P F P K D C V Q M K D I F Y S L L A S L A A A
 R F L R I V F R . K I F F I R C W L V W R P

IGF-8

GACTACAAAG ACGCGTTCG GGCTGCTGTT GCTCCTTGGG GGTGATGAGC CTTTTATGG GCTTCTCCGT
 D Y K D A V A A A V A O W G . . A F L W A S P Y
 T T K T R L R L L L L L G G D E P F Y G L L R
 L Q R R G C G C C C S L G V M S L F H G F S V

ATGCTGATTG GTCGTGGGTC TGCGGCCGCA 100
 A D W S W V C G R
 H L I G R G S A A A
 C . L V V G L R P

IGF-G5

GACTACAAAG ACTGGTTGGT TTGCTTGGGT GTGATGATTA GCTTTTCTG TTAGGGGGT CCGTGTGGCT
 D Y K D W L V C L G V M I S F F C L G G R C G F
 T T K T G W F A W V . . L A F S V Q G V G V A
 L Q R L V G L L G C D D Q L F L F R G S V W L

TTTTACTCAG CGTTGGCTGC CTTGTTGTGT GCCCATAGTG CTTCTTTGGT GTGTGGTGCG GCGGCCGCA 139
 L L S V G C L V V C P Q C F F G V W C G G R
 F Y S A L A A L L C A H S A S L V C G A A A A
 F T Q R W L P C C V P I V L L W C V V R R P

IGF-7

GACTACAAAG ACCCGGATTG GGTGTTGTAG CTGATTAGTT TGGGGTTGGA GGGGATGTAG ATTGGCTGAT
 D Y K D P D W V L Q L I S L G L E G M Q I G . W
 T T K T R I G C C S . L V W G W R G C R L A D
 L Q R P G L G V V A D Q F G V G G D V D W L M

GGGTTTTATG CGTTTTTGAT GGCGC1GGCT GGGGCGGCCG CA 112
 V L C V F D G A G W G G R
 G F Y A F L M A L A G A A A
 G F M R F . W R W L G R P

FIG. 19

Class I Clones		# Clones		Compet-	
		Rnd 3	Rnd 4	ition	
B6 3x	DYKDAETPAQVGNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	1	2	+	
E5 2x	DYKDRHLTNAELGVQSPEVLSRLFPDGDIFYRALSHLVGMGPP	2		+	
B5 2x	DYKDRGGMDRQWLDVGARHLERRSVQDNTDDFYGGLRIIVDGF	2		nd	
9	DYKDGPPDSFDVTEKGDMAILNVRFPDPSLDFNDQTFYFLDDSL		1	nd	
G6	DYKDGTTYFRGQVAQSNESLLRVNFIQLLEALAAASPPT		1	nd	
12	DYKDAPFDARLSAPRFQWSPRTWXQSLSYGEWSCGSFYDCLSSI		1	nd	
A5	DYKDMGSSQFQDTRPSSGQAYSHSLSDSGWTANWIFLRALEGL		1	nd	
C6	DYKDSGAHEGNQGRERSTHLAANINDHLPDAGIWLGYSWLS		1	+	
Consensus (regular+frameshifters) d.FY.lIsaL					
Human IGF mature					
Human IGF propeptide					
Class I frameshifting clones (all in +1 frame)					
F6 2x	TTKTRG.IFGMLLGVLRFQILLWPFPKDCVQMKDIFYSLIAA ^S SL	1	1	+	
7 3x	TTKTRIGCCS.LVWGWRCRLADGFYAFIM ²³ LAG		3	nd	
8	TTKTRLRLLLLGGDEPFYGLLRMLIGRGS		1	nd	
G5	TTKTGWFAWV..LAFSVQGVHVAFYSAIAAALLCAHSASLVCGA	1		nd	
Class II clones					
D5	DYKDPLYGGGIHLYPGTMGYVGFPRQVKVLGDADKNFYDWF ^S M	1		nd	
A6	DYKDYRGMLVLCRISDGACKVASEPPARIGQKVFAVNFYDWFV	1		nd	
R35	DYKDSGCCRLGLRWMFIVIVGWSGALVCQSAASAAGFYDWFV	1		nd	
human IGF mature (1-70)					
GPETLCGAEL VDALQFVCGD RGFYFNKPTG YGSSRRRA ^S PQ TGI ^S VECCFR SCDLRRLEMY CAPLKPAKSA					

nd = no data

FIG. 18

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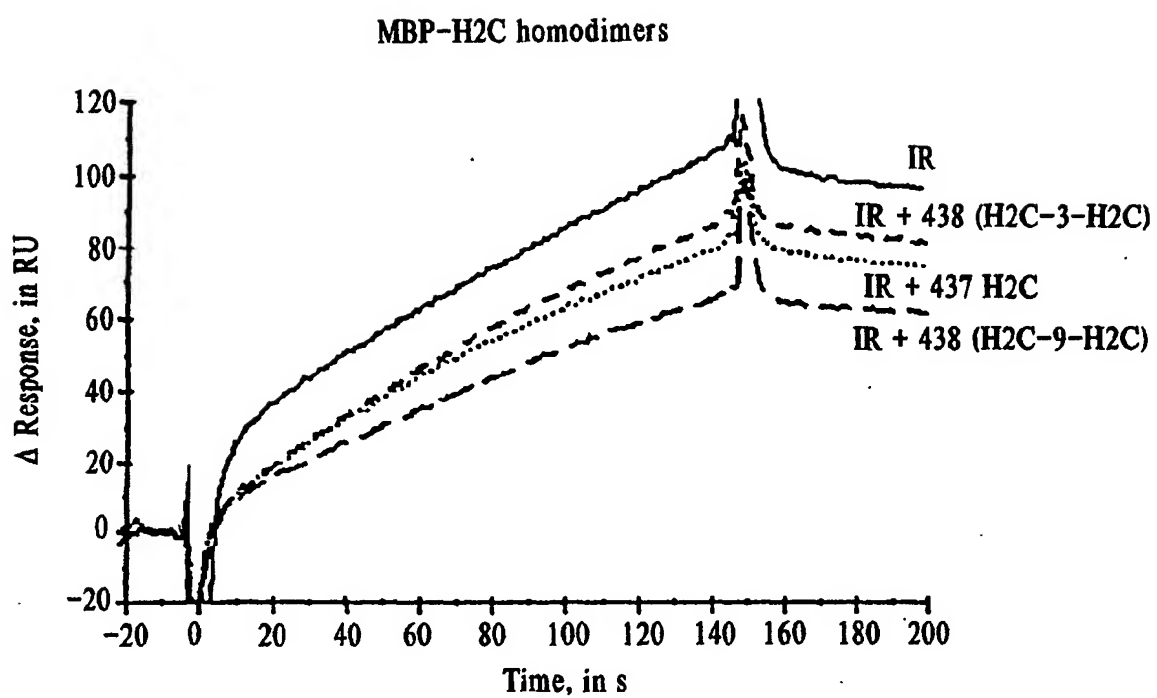


FIG. 17

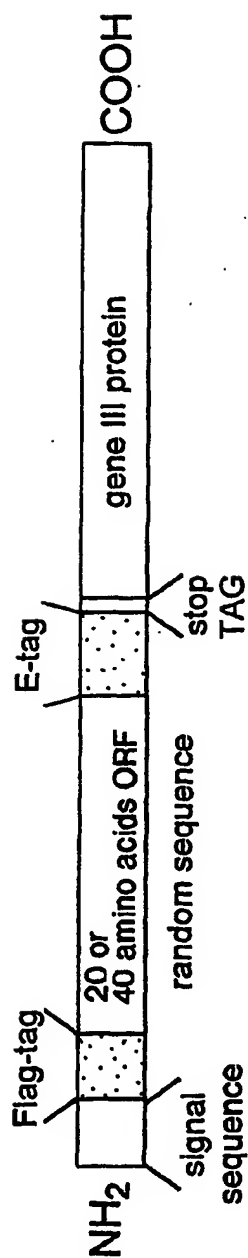


FIG. 16

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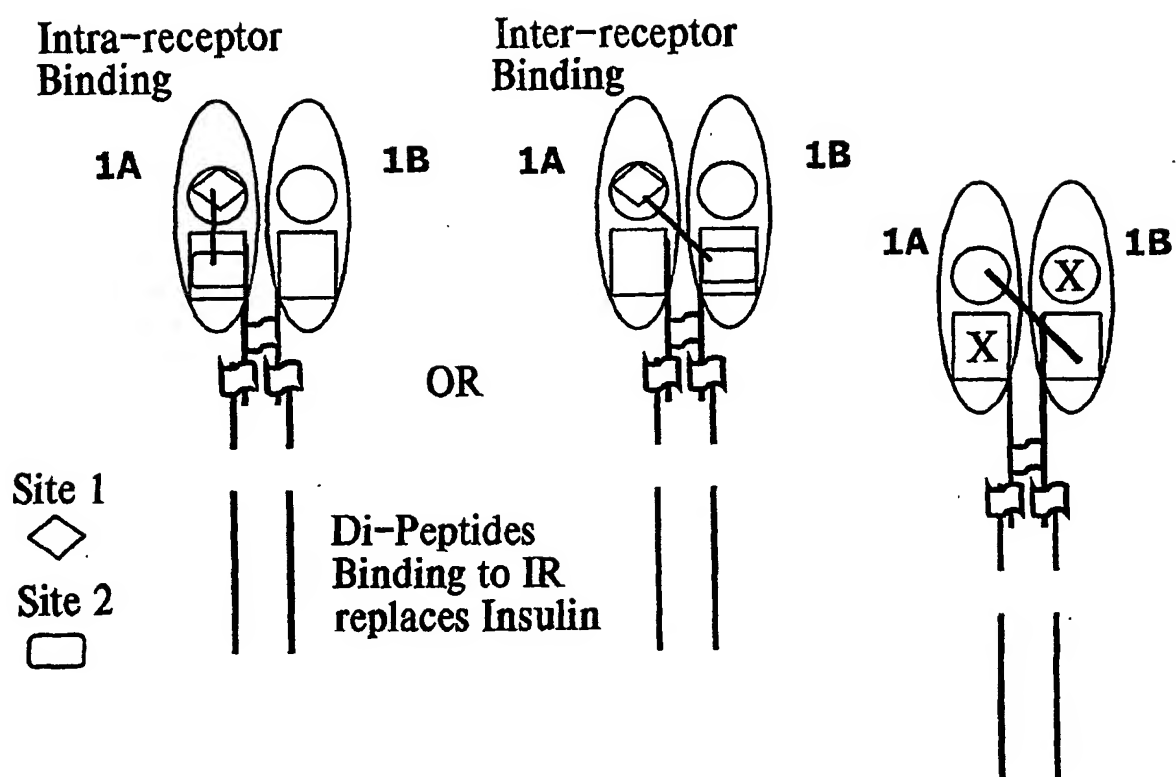


FIG. 15

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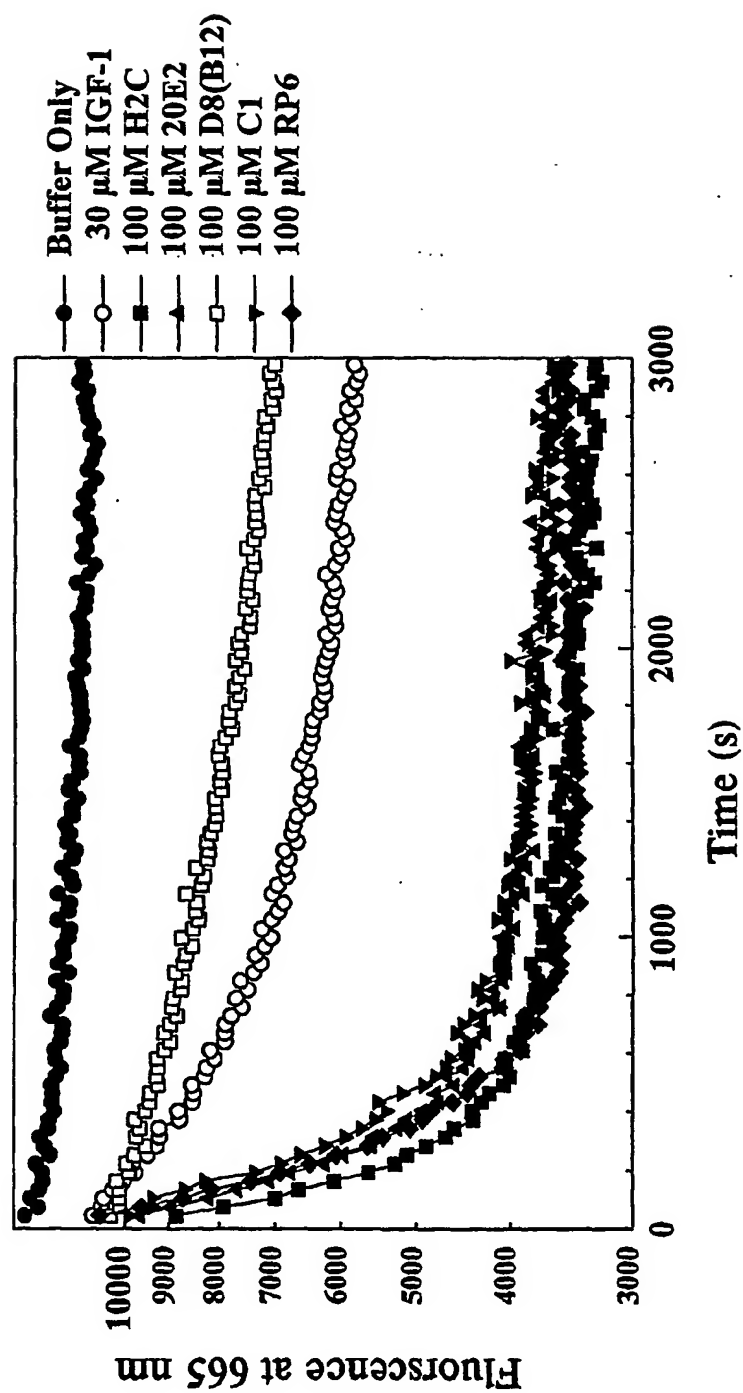


FIG. 14

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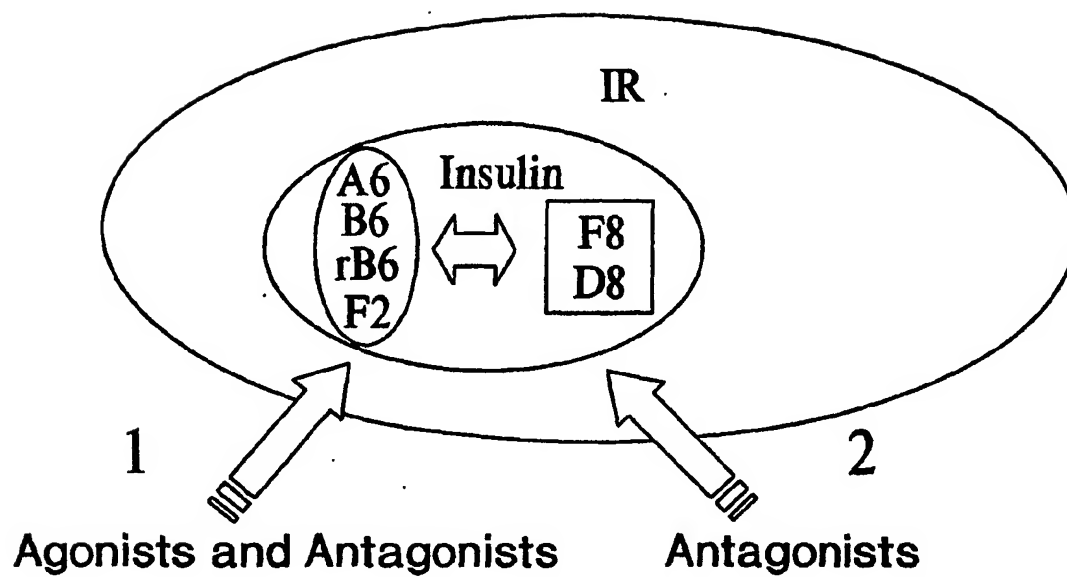
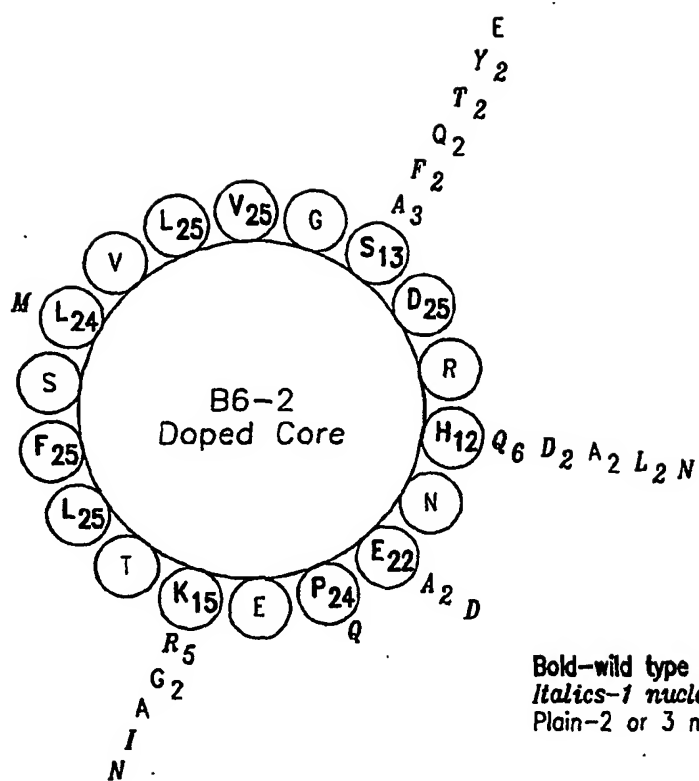


FIG. 13

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Bold—wild type amino acid
Italics—1 nucleotide required to change aa
 Plain—2 or 3 nucleotide required to change aa

FIG. 12

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Clonal Name	D or S name	Motif	Sequence	IR-Kd	IR-IC50 Biacore	IR-IC50FP-S175	P04	Fat Cell Assay
S177	S177	B6	EHWNTVDPPYFTLFEWLRESG	2.7 μ M				+
S178	S178	B6	EHWNTVDPPYQYFSELLRESG	130 nM				0
S179	S179	A6	QSDSGIVHDPFYGWFRDTWAS	540 nM				++
F8-C12	S224	C-C LOOP	FQSLLEELWGCAPLERYGIG					
S225	S225	C-C LOOP	PLCVLEELFWGASLFGTCSG					
D8-G1	S226	C-C LOOP	QLEEEWAGVQCEYVGRECPSS					
S264	S264	A6	IQGWEPFYGMFDDWQAQMFEE					
S257	S257	B6	RWPNFYGYFESLLTHFS					
S258	S258	B6	HYNAFYEFQVLLAETW					
S259	S259	B6	EGWDFYSTFSGLIASVT					

FIG. 11C

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Clonal Name	D or S name	Motif	Sequence	IR-Kd	IR-IC ₅₀ Biotin	IR-IC ₅₀ P-5175	P04	Fat Cell Assay
20-E2	D118	B6	DYKDFYDAIDQLVRSARAGGTRDK K-biotin	250 nM		2.8 nM	+	++
C1	D112	A6	DYKDCWAPCGDAANFYDWFVQOAS KK-biotin	490 nM			-	0
D8	D123	C-C LOOP	KWLDGEAWVQCEVGRCCPSKK	550 nM			0	-
E8	D120	GROUP 6	KRGFGGIVWPGYEWLRNA	370 nM			-	-
F8	D124	C-C LOOP	KHLGVLEELFWGASLFGTCSGKK	40 nM			-	0
H2C	D117	A6	FHENFYDHFVROQVSKK	700 nM	>5 μ M	5 nM	++	++
KCF9			RLTYEWEWGQLEAGRGGLS					
KC-G2		C-C-C	GLEQCCPWGLEVOGRGCP					
KCG7		B6	FYCGLEELSWGALFGYCSG		>1 μ M			
NG-C2		B6	GNGDMFYQLLSLLVGRDMH					
NG-G33		A6	GHSQCPSESYDWFAGOVSDPWGCV		2-4 μ M	4.2 nM	+++	
NG-G8		B6	VEGRGLFYQLRLARRQNG		>5 μ M		-	
NG-G9		B6	RANSFYDALWLGPKK-Biotin				-	
RP-1		A6	GSRPVHEQFYENFVDQLGL		1 μ M		+	
RP-2		A6	RSEASHVEEYSWFEEQLRS		1 μ M		+	
RP-3		A6	GRFYGFQDAIDQLMPWGF		>10 μ M		-	
RP-4		B6	PPWGARFYDATEQLVF		5 μ M		+	
RP-5		B6	AGVNAQFYRYESTLLDWDGKK-Biotin		6 μ M		-	
RP-6		B6+	IFYSCIASLLTGTPOPNRGPWERCCK-Biotin				+++++	
RP-7		A6	AAVHEQFYDWFADQYK					
RP-8		B6	QSFYDYEELGGEWK		>5 μ M		+	
RP-8#	S287	B6	QSFYDYEELGGEWE					
RP-9		A6	GSIDSESYDWFEROIGKK			2.9 nM	++	
RP-10		B6	GSFYALORLVGEGQKK		>10 μ M		+	
RP-11		A6	QAPSNFYDWFVREWDKK		>10 μ M		+	
RP-12		B6	DPFYQGLWEVLRSGKK					
RP-13		A6	ASGFPENFYDWFGRQLSLKK		>10 μ M			
RP-14		A6	SACOFDCHENFYDWFARQKK		>10 μ M			
RP-15		A6	SOAGSAFYAWFDQVLRVKK					
RP-16		B6	V DARDDEUL-SE-VILL					
RP-17		B6	QSDAFYSLGWLIGLSDGKK		>10 μ M			
RP-18		B6	LQPCSGFYDWFQRLHLSGKK					
RP-19		A6	LKDGFDYFQRLHLSGKK					
RP-20		B6	GSASFYDAIDRLRMRIKK					
RP-24		GROUP 6	WPGYLFEEALQDWRGSTD					
S167	S167	A6	AFYDWFACK	>20 μ M	No Binding			
S173	S173	RB6	LDADRLRMRYFERPSL	1.2 μ M				
S174	S174	RB6	PIAELWAYFEHSEGRSSAH	16 μ M				0
S175	S175	A6	GRVDWQRNANFYDWFVLELG	230 nM	2-4 μ M	0.9 nM	++	0
S176	S176	A6	NGVERAGIGDNFYDWFVAQLH	470 nM				+++

FIG. 11B

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D Name	Clonal Name	Formula #	K ₄ (μM) HIR	PO ₄	Fat Cell Assay	Activity	K ₄ (μM) HIGFR	Ratio IGF/IR	Sequence
D101	20D3	1	0.51 0.27				13 11	25 41	KIGGQGHQDGNFYDFWFEALAKK (ε-biotin)
D102	20D1	1	1.2 0.97				7.4 16	6.2 16	KVLQARHGCDSDCFYEWFAKK (ε-biotin)
D103	B8	1	0.74				15	20	KWSALLSYMDTGFYAWFDDAVKK (ε-biotin)
D104	E7	1	20				>20	>1	KGSHWALVRHVDRLFYEWFDLKK (ε-biotin)
D105	H8	1	2.8				12	4.3	KRDKPTDQEEQNWSFYEFRHKK (ε-biotin)
D106	20F1	1	0.97				6.2	6.4	KVFWNCRSQQLDFYEWFEQAOKK (ε-biotin)
D107	40G11	1	1.1	YES		Antagonist	9.7	8.8	KLESHYWPQALDRLFYSWFSKK (ε-biotin)
D108	3G11	1	2.3			Antagonist	19	8.3	KFYGFWSRQLSLTPRDDWGLPKK (ε-biotin)
D109	20H1	1	3.6			Antagonist	12	3.3	KSAPGLVSNKDDGLFYSWFREKK (ε-biotin)
D110	G3	1	0.84			Antagonist	1.4	1.7	KRGGTFYWFESALRKHCAGKK (ε-biotin)
D111	D2	1	0.62				3.2	5.2	KPERMOSDVGFYEFWFRMAVGKK (ε-biotin)
D112	IGFR C1	1	0.49			Neutral	0.05*	0.1	DYKDCWARPCGDAANFYDFWVQQASKK (ε-biotin)
D113	A65-4-C1	1	0.19				0.02*	0.1	
D114	IGFR H2	1	0.75		-20 μM	Agonist	5.4	7.2	DYKDVFTSAHFENFYDFWFRQVSKK (ε-biotin)
D115	IGFR A6	1	8.1			Neutral	>20	>2.5	SAKNFYDFWFKK (ε-biotin)
D116	IGFR D5	1	8.1				>20	>2.5	ADKNFYDFWMAAKK (ε-biotin)
D117	IGFR JBAS	9	4.4 cycli		>20 μM	Agonist	8.1	1.8	DYKDLCSQSGWGRIGWLGLCPKK (ε-biotin)
D118	IGFR H2C	1	0.70	YES	-20 μM	Agonist	6.1	8.6	FHENFYDFWFRQVSKK (ε-biotin)
D119	20E2	2	0.25	YES	-20 μM	Antagonist	5.1	8.5	
D120	20C11	2	0.25	YES	-20 μM	Antagonist	1.3	5.2	DYKDFYDAIDQLVRGSARAGGTRDKK (ε-biotin)
D121	E8	10	0.37				13	2.9	KDRAFYNGLRDLVGAVYGAWDKK (ε-biotin)
D122	F2	10	1.1			Antagonist	2.5	0.8	
D123	20A4 (A7)	6	1.2			Antagonist	2.2	5.9	KVRGFQGGTWPFGYEWLNAKK (ε-biotin)
D124	D8	6	0.55			Antagonist	7.4	6.7	KSMFVAGSDRWPCGYVLADWLKK (ε-biotin)
D125	F8	4	0.04*			Antagonist	>20	>17	KEIEAEWGRVRLYGRVCYGGKK (ε-biotin)
D126	IGFR E4	1	0.09*				>20	>20	
D127	IGFR D2C	1	2.6			Antagonist	>20	>200	KWLDQEWAWVQCEYVGRGCPSSKK (ε-biotin)
D128		1	1.4				16	29	KHLVLEELFWGASLFGYCSGKK (ε-biotin)
D129		1	1.4				>20	>15	
D130		1	1.4				8.2	200	KHLVLEELFWGASLFGYCSGKK (ε-biotin)
D131		1	1.4				>20	>200	
D132		1	1.4				>20	>8	DYKDESAAGTRGNFYDFWFAQVNNKK (ε-biotin)
D133		1	1.4				18	13	LGENFYDFWFMQVRKK

FIG. 11A

Cl ne	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	NNKH-2-C5-IGFR	HL*VLEEL*LGASLFGQWAG	5.4	1.0	2.1	0.5	2.1
	NNKH-2-D9-IGFR	HL*VLEEL*LGASLFGQWAG	7.3	0.9	0.7	1.3	0.8
	NNKH-2-H12-IGFR	HL*VLEEL*LGASLFGQWAG	4.1	0.5	0.4	1.3	0.8
	NNKH-2-D10-IGFR	HL*VLEEL*LGASLFGQWAG	5.0	1.3	1.1	1.2	0.8
	NNKH-2-G9-IGFR	HL*VLEEL*LGASLFGQWAG	4.8	2.1	1.9	1.1	0.9
	NNKH-2-C6-IGFR	HL*VLEEL*LGASLFGQWAG	1.9	1.4	1.3	1.1	0.9
	NNKH-2-C7-IGFR	HL*VLEEL*LGASLFGQWAG	18.2	1.0	0.9	1.1	0.9
	NNKH-2-F11-IGFR	HL*VLEEL*LGASLFGQWAG	21.8	1.3	1.3	1.0	1.0
	NNKH-2-H3-IGFR	HL*VLEEL*LGASLFGQWAG	6.7	1.3	1.4	0.9	1.1
	NNKH-2-B8-IGFR	HL*VLEEL*LGASLFGQWAG	4.7	0.7	0.8	0.9	1.1
	NNKH-2-B12-IGFR	HL*VLEEL*LGASLFGQWAG	17.5	3.7	5.2	0.7	1.4
	NNKH-2-F9-IGFR	HL*VLEEL*LGASLFGQWAG	1.2	1.0	2.9	0.3	2.9
		HL*VLEEL*LGASLFGQWAG	3.7	0.6	2.1	0.3	3.5

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FIG. 10I

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
F815-4-G11-IGFR		<u>HLCVLEELFWGASLFGYCSG</u>	39.1	1.8	27.7	0.1	15.4	
F815-3-D1-IGFR		HFYVLVERLSGASLFGSGSA	34.6	7.9	1.0	7.9	0.1	
F815-4-C12-IGFR		HRFVREGLLWGAYQFCYCSG	14.9	1.0	2.0	0.5	2.0	
F815-4-A11-IGFR		FQSLLEELVWGAPLFRYGTG	35.2	1.0	2.0	0.5	2.0	
		HLSVLEELSWGASLFGQWAG	5.4	1.0	2.1	0.5	2.1	

FIG. 10G

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
NNKH-4-A9-IR		<u>HLSVLEELSWGASLFGQWAG</u>	5.4	1.0	2.1	0.5	2.1	
NNKH-4-H4-IR		NLCRLEELANGASLFGQCAG	16.3	1.0	2.7	0.4	2.6	
NNKH-4-B3-IR		APVSTEELRWGALLFGQWAG	15.6	1.0	2.6	0.4	2.5	
NNKH-4-E1-IR		HLSVLEERWWRRESLFGQWAG	13.6	2.8	6.7	0.4	2.3	
NNKH-4-E7-IR		HLSVLEERWWRRAALFGQWAG	13.9	4.8	9.5	0.5	2.0	
NNKH-4-G3-IR		HLSILEEQWWRRESLFGQWAG	16.9	1.3	2.3	0.6	1.8	
NNKH-4-B6-IR		HMSVEELSWWASLFGKQAG	11.3	1.3	2.3	0.6	1.7	
NNKH-4-A10-IR		HLSLEERWWRATLFGQWAG	13.2	1.3	2.1	0.6	1.7	
NNKH-4-A5-IR		HLSVLEELWWRRESLFGQWAG	15.4	2.0	3.2	0.6	1.6	
NNKH-4-F11-IR		HLSLLEEQWWRRESLFGQWAG	14.6	4.6	6.9	0.7	1.5	
NNKH-4-C9-IR		HLSVLEERWWRRESLFGQWAG	14.0	3.1	3.9	0.8	1.3	
NNKH-4-D12-IR		HLSVLEEQWWRRESLFGQWAG	14.3	2.3	2.9	0.8	1.3	
NNKH-4-D10-IR		HLSVLEEQW.ESLFGQWAG	12.0	1.4	1.7	0.8	1.2	
NNKH-4-E5-IR		HLSVLEELWWRRESLFGQWAG	13.6	1.2	1.5	0.8	1.2	
NNKH-2-A6-IR		HLSVLEERWWRATLFGQWAG	14.5	1.4	1.6	0.9	1.1	
NNKH-4-F6-IR		HL.VLEELLWVSLFRQWAG	8.4	1.4	1.5	1.0	1.1	
NNKH-4-C7-IR		HLSALEEQWWRATLFGQWAG	14.1	2.8	2.9	1.0	1.0	
NNKH-4-F7-IR		HLSVLEERWWRATLLESQ	14.7	1.4	1.4	1.0	1.0	
NNKH-4-F8-IR		HLSALEELWWRRESLFGQWAG	14.1	7.5	7.0	1.1	0.9	
NNKH-4-E9-IR		HLSVLEELWWRRESLFGKQAG	13.6	11.0	8.6	1.3	0.8	
NNKH-4-E6-IR		HLSVLEEAWWRESLFGHWAG	15.5	7.9	6.0	1.3	0.8	
NNKH-4-B7-IR		HMSVEELWWRATLFGQWAG	18.2	3.8	2.7	1.4	0.7	
NNKH-2-B3-IR		HLSVLEERWWRRESLFGQWAG	16.5	12.9	8.2	1.7	0.6	
		HRSVLKQLSWGASLFGQWAG	11.5	5.3	0.7	7.4	0.1	

FIG. 10H

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
NNKH-4-A9-IR		<u>HLSVLEELSWGASLFGQWAG</u>	5.4	1.0	2.1	0.5	2.1	
NNKH-4-H4-IR		NLCRLEELANGASLFGQCAG	16.3	1.0	2.7	0.4	2.6	
NNKH-4-B3-IR		APVSTEELRWGALLFGQWAG	15.6	1.0	2.6	0.4	2.5	
NNKH-4-E1-IR		HLSVLEERWWRRESLFGQWAG	13.6	2.8	6.7	0.4	2.3	
NNKH-4-E7-IR		HLSVLEERWWRRAALFGQWAG	13.9	4.8	9.5	0.5	2.0	
NNKH-4-G3-IR		HLSILEEQWWRRESLFGQWAG	16.9	1.3	2.3	0.6	1.8	
NNKH-4-B6-IR		HMSVEELSWWASLFGKQAG	11.3	1.3	2.3	0.6	1.7	
NNKH-4-A10-IR		HLSLEERWWRATLFGQWAG	13.2	1.3	2.1	0.6	1.7	
NNKH-4-A5-IR		HLSVLEELWWRRESLFGQWAG	15.4	2.0	3.2	0.6	1.6	
NNKH-4-F11-IR		HLSLLEEQWWRRESLFGQWAG	14.6	4.6	6.9	0.7	1.5	
NNKH-4-C9-IR		HLSVLEERWWRRESLFGQWAG	14.0	3.1	3.9	0.8	1.3	
NNKH-4-D12-IR		HLSVLEEQWWRRESLFGQWAG	14.3	2.3	2.9	0.8	1.3	
NNKH-4-D10-IR		HLSVLEEQW.ESLFGQWAG	12.0	1.4	1.7	0.8	1.2	
NNKH-4-E5-IR		HLSVLEELWWRRESLFGQWAG	13.6	1.2	1.5	0.8	1.2	
NNKH-2-A6-IR		HLSVLEERWWRATLFGQWAG	14.5	1.4	1.6	0.9	1.1	
NNKH-4-F6-IR		HL.VLEELLWVSLFRQWAG	8.4	1.4	1.5	1.0	1.1	
NNKH-4-C7-IR		HLSALEEQWWRATLFGQWAG	14.1	2.8	2.9	1.0	1.0	
NNKH-4-F7-IR		HLSVLEERWWRATLLESQ	14.7	1.4	1.4	1.0	1.0	
NNKH-4-F8-IR		HLSALEELWWRRESLFGQWAG	14.1	7.5	7.0	1.1	0.9	
NNKH-4-E9-IR		HLSVLEELWWRRESLFGKQAG	13.6	11.0	8.6	1.3	0.8	
NNKH-4-E6-IR		HLSVLEEAWWRESLFGHWAG	15.5	7.9	6.0	1.3	0.8	
NNKH-4-B7-IR		HMSVEELWWRATLFGQWAG	18.2	3.8	2.7	1.4	0.7	
NNKH-2-B3-IR		HLSVLEERWWRRESLFGQWAG	16.5	12.9	8.2	1.7	0.6	
		HRSVLKQLSWGASLFGQWAG	11.5	5.3	0.7	7.4	0.1	

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
20C-3-H3-IGFR	xxxxxxxxxxxxxxxxxxxxxxxxxxxx	--	--	--	--
20C-3-F4-IGFR	DHRLCGTDEYLMQDLFVRGLCRLIW	28.5	26.6	1.0	26.6 <0.1
20C-4-C10-IGFR	GLLFCKQLFTLAGLQPEAGCVSSSR	34.4	27.5	1.2	23.1 <0.1
20C-3-G5-IGFR	IWIACLDELLRGQVWSSCRRRAPIG	35.5	24.4	1.3	19.2 0.1
20C-3-A2-IGFR	DWLRCLGVILSGGLTELANTCVQOG	29.3	21.1	1.1	18.7 0.1
20C-3-B4-IGFR	WFSFCLGGLLQAEWSVWGRDVGCI	33.9	18.3	1.1	16.9 0.1
20C-3-C6-IGFR	GYSWLRDVLMKQAOQLKREGSVGRQ	39.8	29.1	1.9	15.2 0.1
20C-3-E2-IGFR	FLTRLLERLGLS*ERGEAGGPYAQA	34.8	20.9	1.4	14.9 0.1
20C-3-A3-IGFR	FSGFCMGLERLSQVSLGCGAGQGG	34.8	28.1	2.0	14.2 0.1
20C-3-B1-IGFR	ISFRQQLFVLACMHPCVPDVGGEGF	33.7	14.3	1.2	12.4 0.1
20C-3-F5-IGFR	NTPNCSQDWGOESGFMALLALTCK	30.2	9.8	0.9	11.2 0.1
20C-4-A7-IGFR	LQFCCELLATVTGVTGLGCLDYQPI	35.5	31.9	3.9	8.2 0.1
20C-4-F8-IGFR	GSSICNLLARAQIVELALCEMGVQE	33.3	19.3	2.8	6.9 0.1
20C-4-G11-IGFR	LSFACLLSLSLGVLPDCLLGED	30.5	27.7	5.3	5.2 0.2
20C-3-E1-IGFR	GEHFCQLLMSLCGDDCGPVCNCGGS	24.7	13.3	2.8	4.7 0.2
20C-3-B6-IGFR	GWFECLLASLVLPQGRSRASAVC	34.0	5.1	1.6	3.1 0.3
	YRQECACSVGAVGFLCGLACLARSG	37.3	32.8	13.7	2.4 0.4

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FIG. 10F-1

Clone Design	Sequence
20C-3-H3-IGFR	xxxxxxxxxxxxxxxxxxxxxxxxxxxx
20C-3-F4-IGFR	DHRLCGTDEYLMQDLFVRGLCRLIW
20C-4-C10-IGFR	GLLFCKQLFTLAGLQPEAGCVSSSR
20C-3-G5-IGFR	IWIACLDELLRGQVWSSCRRRAPIG
20C-3-A2-IGFR	DWLRCLGVILSGGLTELANTCVQOG
20C-3-B4-IGFR	WFSFCLGGLLQAEWSVWGRDVGCI
20C-3-C6-IGFR	GYSWLRDVLMKQAOQLKREGSVGRQ
20C-3-E2-IGFR	FLTRLLERLGLS*ERGEAGGPYAQA
20C-3-A3-IGFR	FSGFCMGLERLSQVSLGCGAGQGG
20C-3-B1-IGFR	ISFRQQLFVLACMHPCVPDVGGEGF
20C-3-F5-IGFR	NTPNCSQDWGOESGFMALLALTCK
20C-4-A7-IGFR	LQFCCELLATVTGVTGLGCLDYQPI
20C-4-F8-IGFR	GSSICNLLARAQIVELALCEMGVQE
20C-4-G11-IGFR	LSFACLLSLSLGVLPDCLLGED
20C-3-E1-IGFR	GEHFCQLLMSLCGDDCGPVCNCGGS
20C-3-B6-IGFR	GWFECLLASLVLPQGRSRASAVC
	YRQECACSVGAVGFLCGLACLARSG

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
40F-4-D1-IGFR	xxxxxxxxxxxxxxxxxxxxxxxxxxxx	--	--	--	--
40F-4-B1-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	4.9	4.6	0.3	13.1 0.1
40F-4-D10-IGFR	GLDHSDAVGVLHGFAPWA.ARGWRWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1 0.1
40F-3-A3-IGFR	W.GYAWLS	4.9	4.5	0.4	11.7 0.1
40F-4-C4-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	2.6	2.0	0.3	7.9 0.1
	EAMAVGLQCPARFVRAAAHGDGGSWGQDHV.AWGGYWWLG	3.8	2.0	0.5	4.1 0.2

FIG. 10F-2

Clone Design	Sequence
40F-4-D1-IGFR	xxxxxxxxxxxxxxxxxxxxxxxxxxxx
40F-4-B1-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE
40F-4-D10-IGFR	GLDHSDAVGVLHGFAPWA.ARGWRWEAGGLEDTWAGYDWL
40F-3-A3-IGFR	W.GYAWLS
40F-4-C4-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE
	EAMAVGLQCPARFVRAAAHGDGGSWGQDHV.AWGGYWWLG

Ratios over Background			Comparisons	
E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
--	--	--	--	--
44.6	1.5	2.7	0.6	1.8
46.4	1.9	2.1	0.9	1.1

Sequence
RGTRTDLRLKSGGFAIVPRWPFCFSYHCLVEMITKTGSPG
GRTSMAFVPPRHQLPELAPRPNRHAWLVGG

FIG. 10D

Ratios over Background			Comparisons	
E-Tag	IGFsR	IR	IGF/IR	IR/IGF
--	--	--	--	--
35.5	6.0	--	--	--
29.1	4.7	--	--	--
25.7	3.0	--	--	--
15.6	2.7	--	--	--

Sequence
XXXXXXXXXXXXXXXXXXXX
CLGAGSFRAGILCLGGLPVS
GFWATACGGGLQICEELGLKP
DLFCAYMAQALGLGQDLSCG
RHLLLPQIWIAS*GGWGMG

FIG. 10E

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	IR/IGFR
R20 β -4-A4-IR	WPGYLFEEALQDWRGSTD	11.9	17.5	1.4	12.5	0.1	0.1
R20 β -4-F2-IR	SMFVAGSDRWPGYGVLDWL	16.4	13.9	3.1	4.5	0.2	0.2
R20 β -4-E8-IR	VRGFQGGTVWPGYEWLRNAA	41.0	34.9	3.6	9.7	0.1	0.1

FIG. 10B-1

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	IR/IGFR
20F-4-H10-IR	LDLASGDSWLGYDVLRGWLS	10.2	3.1	2.4	1.3	0.8	0.8
20F-4-C10-IR	IHSSDGIGAWGGYAWFRDVA	23.4	9.6	4.1	2.3	0.4	0.4

FIG. 10B-2

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Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	IR/IGFR
R20 β -4-D10-IR	LGPLLRWGSEVCGVWPDICE	21.5	1.0	8.0	0.1	8.0	8.0
R20 β -4-D9b-IR	PFGEGRWGWGIPRMWYRNS	32.6	6.8	15.1	0.5	2.2	2.2
R20 β -4-H4-IR	WWWGGRNRWWLERWGLGGER	11.6	1.7	3.6	0.5	2.1	2.1
R20 β -4-A2-IR	GRVALWGPVWPRWWFMSRPV	17.1	2.6	5.2	0.5	2.0	2.0

FIG. 10C

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
R20-4-C10-IGFR	XXXXXXXXXXXXXXXXXXXXX PKGTRFRGDVVDVWDGYSWLA	--	--	--	--
		37.8	3.8	--	--

FIG. 10A--1

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
20F-4-B7-IGFR	XXXXXXXXXXXXXXXXXXXXX TPIPAGGINLASWGGYTWLS	--	--	--	--
20F-4-E4-IGFR		10.9	3.7	7.3	0.1
20F-4-E12-IGFR		8.9	4.7	6.3	0.2
20F-4-F4-IGFR		9.7	4.7	6.0	0.2
20F-4-F7-IGFR		13.9	10.1	5.6	0.2
20F-4-E7-IGFR		13.7	3.9	5.1	0.2
20F-4-F11-IGFR		7.2	2.5	4.7	0.2
20F-4-D10-IGFR		17.6	16.2	4.6	0.2
20F-4-B3-IGFR		9.8	2.4	4.1	0.2
20F-4-B12-IGFR		17.3	14.4	4.0	0.2
20F-3-A9-IGFR		10.1	9.9	4.0	0.2
20F-4-G2-IGFR		6.6	2.7	4.0	0.2
20F-4-D11-IGFR		5.1	1.3	2.7	0.4
20F-4-G4-IGFR		5.0	1.0	2.3	0.4
20F-4-G12-IGFR		3.9	0.9	1.8	0.6
		3.2	0.9	1.5	0.7

FIG. 10A--2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGF/IR	IR/IGF
JBA5-4-G12-IR	LCQSWGVRIQWLAGLCP	37.5	3.0	1.4	0.5
JBA5-4-G3-IR	LCQSWDACIQWLVGLSP	4.5	2.5	1.1	0.4
JBA5-4-G1-IR	LCRSWEECIGWLVGPQP	32.0	3.2	1.3	0.4
JBA5-3-B1-IR	LCQSWGECIDRLVGQGA	29.4	6.8	1.2	0.2
JBA5-3-C1-IR	LCQGWGVRIQWLAGLCP	36.3	7.5	1.1	0.1
JBA5-3-A6-IR	LCQGWAVHIGQLAGLCP	28.0	7.4	0.7	0.1
JBA5-3-A2-IR	LCQGWGVHIGRLAGLCP	10.2	4.8	0.4	0.1
JBA5-3-B7-IR	LCQSWGVHIGRLAGLCP	39.2	15.2	1.2	0.1

FIG. 9C

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Clon	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
H5 Parental	LCQSLGVTPGWLAGNCA	--	1.2	--	--
H5-3-JBA5-IGFR	LCQSWGVRIGWLAGLCP	31.9	16.3	--	--
H5-3-E1A11-IGFR	VCQSLGITDLGICAGNGA	21.3	8.0	--	--
H5-3-E4B10-IGFR	LCQSLGLTHPGFEAWLCA	29.7	7.8	--	--
H5-3-E4C10-IGFR	LCQNFVTDPGCFYGFWA	24.3	6.1	--	--
H5-3-JBB6-IGFR	PCQRLGDTHLCWLAGWEA	40.2	5.4	--	--
H5-3-E4A9-IGFR	LCQSSGLSFLGCLGWWA	27.7	4.3	--	--
H5-3-E2A12-IGFR	LCQSLGFTDLDWLACWFE	27.2	4.2	--	--
H5-3-E4A12-IGFR	VCQGLGVECPGWFAAGWWA	27.9	3.9	--	--
H5-3-E1F9-IGFR	PCQSLGLTCSGNFEGWGA	18.6	3.5	--	--
H5-3-E4F11-IGFR	LCQGWGIRIGWLVGRCM	28.4	3.3	--	--
H5-3-E4A11-IGFR	LWQSVGIKYPGGLAGWLA	31.0	3.0	--	--
H5-3-E4G7-IGFR	QWQSLGVTCPGSWAELCA	26.2	2.2	--	--
H5-3-E1B9-IGFR	LCQSLGVTYWEGLAWLCA	20.0	2.1	--	--

FIG. 9A

Clon	Sequence
H5 Parental	LCQSLGVTPGWLAGNCA
H5-3-JBA5-IGFR	LCQSWGVRIGWLAGLCP
H5-3-E1A11-IGFR	VCQSLGITDLGICAGNGA
H5-3-E4B10-IGFR	LCQSLGLTHPGFEAWLCA
H5-3-E4C10-IGFR	LCQNFVTDPGCFYGFWA
H5-3-JBB6-IGFR	PCQRLGDTHLCWLAGWEA
H5-3-E4A9-IGFR	LCQSSGLSFLGCLGWWA
H5-3-E2A12-IGFR	LCQSLGFTDLDWLACWFE
H5-3-E4A12-IGFR	VCQGLGVECPGWFAAGWWA
H5-3-E1F9-IGFR	PCQSLGLTCSGNFEGWGA
H5-3-E4F11-IGFR	LCQGWGIRIGWLVGRCM
H5-3-E4A11-IGFR	LWQSVGIKYPGGLAGWLA
H5-3-E4G7-IGFR	QWQSLGVTCPGSWAELCA
H5-3-E1B9-IGFR	LCQSLGVTYWEGLAWLCA

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
JBA5 Parental	LCQSWGVRIGWLAGLCP	31.5	20.6	1.0	<0.1
JBA5-4-2C12-IGFR	LCQSWGVRIGWLAGLCP	46.8	41.5	1.0	<0.1
JBA5-2-1F9-IGFR	LCESWGVRIGWLAGLCP	48.1	39.5	1.0	<0.1
JBA5-2-1E10-IGFR	LCQSWGVRIGWLVGLCP	42.5	39.5	1.1	<0.1
JBA5-4-2A11-IGFR	LCQGWGVRIGWLAGLCP	44.1	40.2	1.2	<0.1
JBA5-3-2A3-IGFR	LCQSWGVRIGWLVGLCP	34.7	33.3	1.0	<0.1
JBA5-4-2A9-IGFR	LCQSWGVRIGWLVGLCP	34.6	33.1	1.0	<0.1
JBA5-1-1B6-IGFR	MCQSWDVRIGRLGGQCP	39.6	31.4	1.0	<0.1
JBA5-4-2B9-IGFR	LCQGWVVRIGWLAGLCP	39.6	22.3	1.0	<0.1
JBA5-1-1H7-IGFR	LCQGWGVRIGWLAGLCP	24.9	22.6	1.2	0.1
JBA5-3-2C3-IGFR	LCQSWDVRIGWVAGLCP	35.5	15.3	1.1	0.1
JBA5-1-1G7-IGFR	LCQSWDARIGWLAGLCP	26.2	14.8	1.5	0.1
JBA5-2-1E9-IGFR	LCLG*DVRIGLLAGLCP	39.4	4.5	1.0	0.2
JBA5-2-1D12-IGFR	L*KSVDVRSGLMAGLCP	42.2	2.2	1.0	0.5

FIG. 9B

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J216	VCAWPTYWNCG	$2.8 \cdot 10^{-6}$
J227	SFYEAHQLLGV-NH ₂	$6.4 \cdot 10^{-6}$
J228	HPPLEHLKAFLL-NH ₂	$2.4 \cdot 10^{-5}$
J229	APTFYAWFNQQT-NH ₂	$2.4 \cdot 10^{-6}$
S122	HPTSKEIYAKLLK	$9.3 \cdot 10^{-6}$
S123	HPSTNQMLMKLFK	$1.6 \cdot 10^{-5}$
S124	HPPLSELKLFLIKK	$2.3 \cdot 10^{-5}$

FIG. 8-4

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J185	ACV _D PTYWNCG	$3.4 \cdot 10^{-5}$
J186	AC _D WPTYWNCG	$3.5 \cdot 10^{-5}$
J187	ACVWTYWNPCG	$4.3 \cdot 10^{-5}$
J188	ACVWTYWPNCG	$3.0 \cdot 10^{-5}$
J189	ACVWTPWNCG	$3.1 \cdot 10^{-5}$
J190	ACVWTPYWNCG	$2.6 \cdot 10^{-5}$
J191	ACVPWTYWNCG	$3.0 \cdot 10^{-5}$
J192	ACPVWTYWNCG	$4.2 \cdot 10^{-5}$
J193	ACWPTYWNVCG	$4.8 \cdot 10^{-5}$
J194	ACPTYWNVWCG	$4.2 \cdot 10^{-5}$
J195	ACTYWNVWPCG	$3.3 \cdot 10^{-5}$
J196	ACYWNVWPTCG	$2.4 \cdot 10^{-5}$
J197	ACWNVWPTYCG	$2.9 \cdot 10^{-5}$
J198	ACNVWPTYWCG	$4.2 \cdot 10^{-5}$
J199	ACVWPCG	$4.7 \cdot 10^{-5}$
J200	CVWPTYWNCG	$5.5 \cdot 10^{-5}$
J201	ACWWPTYWNCG	$6.8 \cdot 10^{-6}$
J202	ACEWPTYWNCG	$4.6 \cdot 10^{-6}$
J203	ACRWPTYWNCG	$5.8 \cdot 10^{-6}$
J204	ACQWPTYWNCG	$9.2 \cdot 10^{-6}$
J205	ACGWPTYWNCG	$4.4 \cdot 10^{-6}$
J207	cyclo-Valeroyl-AWPTYWNCG	$5.5 \cdot 10^{-5}$
J208	cyclo-Toluy- AWPTYWNCG	$7.6 \cdot 10^{-5}$
J209	cyclo-Acetyl- AWPTYWNCG	$7.7 \cdot 10^{-5}$
J210	(WPTYWNCG) ₂	$5.3 \cdot 10^{-5}$
J211	(AWPTYWNCG) ₂	$7.9 \cdot 10^{-6}$
J212	ACA(Bpa)PTYWNCGK(biotin)	$1.8 \cdot 10^{-5}$
J213	ACAWPTY(Bpa)NCGK(biotin)	$1.8 \cdot 10^{-5}$
J214	GCAWPTYWNCG	$1.4 \cdot 10^{-6}$
J215	NCAWPTYWNCG	$9.0 \cdot 10^{-6}$

FIG. 8-3

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J137	AC(dMet)WPTYWNCG	$2.5 \cdot 10^{-5}$
J138	AC(Abu)WPTYWNCG	$7.8 \cdot 10^{-5}$
J139	AC(dAbu)WPTYWNCG	$2.1 \cdot 10^{-5}$
J140	AC(Nva)WPTYWNCG	$3.6 \cdot 10^{-6}$
J141	AC(dNva)WPTYWNCG	$3.0 \cdot 10^{-5}$
J142	AC(tBuG)WPTYWNCG	$3.2 \cdot 10^{-5}$
J143	AC(dtBuG)WPTYWNCG	$3.8 \cdot 10^{-5}$
J144	AC(Phe)WPTYWNCG	$5.1 \cdot 10^{-6}$
J145	AC(dPhe)WPTYWNCG	$5.7 \cdot 10^{-5}$
J146	AC(Cha)WPTYWNCG	$2.2 \cdot 10^{-5}$
J147	AC(dCha)WPTYWNCG	$1.7 \cdot 10^{-5}$
J148	AC(Nal(1))WPTYWNCG	$5.8 \cdot 10^{-6}$
J149	AC(dNal(1))WPTYWNCG	$2.0 \cdot 10^{-5}$
J150	AC(Acy)WPTYWNCG	$2.0 \cdot 10^{-5}$
J151	ACVWPT(Hyp)WNCG	$2.2 \cdot 10^{-4}$
J154	ACVWPT(Nal2)WNCG	$8.2 \cdot 10^{-5}$
J155	ACVWPT(MetO ₂)WNCG	$1.9 \cdot 10^{-4}$
J157	ACVWPT(Cha)WNCG	$1.2 \cdot 10^{-4}$
J160	ACVWPT(Ser)WNCG	$1.8 \cdot 10^{-4}$
J162	ACVWPT(Thi)WNCG	$2.5 \cdot 10^{-4}$
J163	ACVWPT(dSer)WNCG	$5.0 \cdot 10^{-5}$
J166	ACVWPT(dCha)WNCG	$7.5 \cdot 10^{-5}$
J170	ACVWPT(dPhe)WNCG	$1.4 \cdot 10^{-4}$
J171	ACVWPT(Thr)WNCG	$7.7 \cdot 10^{-4}$
J174	ACVWPT(Phe)WNCG	$4.5 \cdot 10^{-5}$
J176	ACVWPT(dThr)WNCG	$2.8 \cdot 10^{-5}$
J180	ACVWPTYW _D CG	$5.6 \cdot 10^{-5}$
J182	ACVWPT _D WNCG	$2.7 \cdot 10^{-5}$
J183	ACVWP _D YWNCG	$3.3 \cdot 10^{-5}$
J184	ACVW _D TYWNCG	$6.2 \cdot 10^{-5}$

FIG. 8-2

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J-nr	Sequence	HIR affinity mol/l
J101	ACVWPTYWNCG	$5.0 \cdot 10^{-6}$
J103	Ac-CVWPTYWNCG	$3.0 \cdot 10^{-5}$
J104	Bz-CVWPTYWNCG	$3.2 \cdot 10^{-5}$
J105	Ac-ACVWPTYWNCG	$4.5 \cdot 10^{-5}$
J109	ACVWPTYWACG	$2.0 \cdot 10^{-5}$
J110	ACVWPTYANCG	$2.4 \cdot 10^{-5}$
J111	ACVWPTAWNCG	$3.1 \cdot 10^{-5}$
J112	ACVWPAYWNCG	$3.3 \cdot 10^{-5}$
J113	ACVWATYWNCG	$5.5 \cdot 10^{-5}$
J115	ACAWPTYWNCG	$2.7 \cdot 10^{-6}$
J116	AAVWPTYWNAG	$3.4 \cdot 10^{-5}$
J117	ASVWPTYWNSG	$2.9 \cdot 10^{-5}$
J118	ACPYNWWTWCG	$2.9 \cdot 10^{-5}$
J119	ACVWPTYW _n CG	$3.2 \cdot 10^{-5}$
J120	ACVWPTYwNCG	$3.4 \cdot 10^{-5}$
J121	ACVWPTYWNCG	$1.8 \cdot 10^{-5}$
J122	ACVWPtYWNCG	$5.1 \cdot 10^{-5}$
J123	ACVWpTYWNCG	$2.5 \cdot 10^{-5}$
J124	ACVwPTYWNCG	$2.0 \cdot 10^{-5}$
J125	ACWPTYWNCG	$1.8 \cdot 10^{-5}$
J127	acvwptywncg	$4.4 \cdot 10^{-5}$
J128	gcnwytpwvca	$5.3 \cdot 10^{-5}$
J130	AEVWPTYWN(Dpr)G	$1.9 \cdot 10^{-5}$
J131	AC _D WPTYWNCG	$5.5 \cdot 10^{-5}$
J132	AC(Leu)WPTYWNCG	$4.5 \cdot 10^{-6}$
J133	AC(dLeu)WPTYWNCG	$2.8 \cdot 10^{-5}$
J134	AC(Ile)WPTYWNCG	$7.4 \cdot 10^{-6}$
J135	AC(dIle)WPTYWNCG	$2.9 \cdot 10^{-5}$
J136	AC(Met)WPTYWNCG	$7.5 \cdot 10^{-6}$

FIG. 8-1

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	Sequence	HIR affinity mol/l
J228	HPPLEHLKAFLL-NH ₂	2.4*10 ⁻⁵
J229	APTFYAWFNQQT-NH ₂	2.4*10 ⁻⁶
S122	HPTSKEIYAKLLK	9.3*10 ⁻⁶
S123	HPSTNQMLMKLFK	1.6*10 ⁻⁵
S124	HPPLSELKLFLIKK	2.3*10 ⁻⁵

FIG. 7

		Ratios over Background		Comparisons	
Clon	Parental/Design	E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
D820-4-G9-IGFR	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1 17.3
D820-4-C10-IGFR	WLDQEWAWVQCEVWGRGCPS	36.8	1.0	29.6	<0.1 29.6
D820-4-A9-IGFR	WLDLEWFEVQCEVYGRGCPT	32.6	1.0	31.3	<0.1 31.3
D820-4-B8-IGFR	WLEQEWASVQCEVYGRGCPS	20.4	1.0	31.4	<0.1 31.4
D820-4-F8-IGFR	WLDLEWEQIKCKVYGRGCPT	31.1	1.0	32.7	<0.1 32.7
D820-4-H7-IGFR	WLEQEWAIQCEVYGRGCPS	28.3	1.0	32.9	<0.1 32.9
D820-4-E8-IGFR	WLEQEWALVLCVYGHGCPA	34.1	1.0	32.9	<0.1 32.9
D820-4-G10-IGFR	WLEQEWAIQCEVWGRGCSS	26.6	1.0	33.2	<0.1 33.2
D820-4-D10-IGFR	WLE?EWENWQCEVYGRGC?S	37.5	1.0	33.2	<0.1 33.2
D820-4-D8-IGFR	WLEQEWAVQCDVYGRGCPS	36.6	1.0	33.5	<0.1 33.5
D820-4-A10-IGFR	WLEQE*ARVQCEVWGRGCPS	23.7	1.0	34.6	<0.1 34.6
D820-4-B7-IGFR	WL?QEWARVHCEVWGRP?QC	29.4	1.0	35.5	<0.1 35.5
D820-4-E12-IGFR	PLEHEWAWVQCVYGRGCPS	35.4	1.0	36.9	<0.1 36.9
D820-4-H10-IGFR	SLE?EWAWVQCEV?GRGCP?	37.0	1.0	37.0	<0.1 37.0
D820-4-F12-IGFR	WLDQEWVRVQCEVWGRGCPS	36.8	1.0	37.1	<0.1 37.1
D820-4-F7-IGFR	SLDKEWAWKCEVYGRGCPS	36.9	1.0	37.3	<0.1 37.3
D820-4-G12-IGFR	LGDQEWAWVEV#GRGWPS	34.4	1.0	37.5	<0.1 37.5
D820-4-D12-IGFR	WLEEEWAIQRCVYGRGCPS	30.3	1.0	37.8	<0.1 37.8
D820-4-A12-IGFR	WLEEE*GWVQCEVWGRGCPP	37.2	1.0	38.6	<0.1 38.6
D820-4-C12-IGFR	CLDQEWAVQCPVYGRGCPS	30.4	1.0	39.3	<0.1 39.3
D820-4-A7-IGFR	QLELEWAVQCEVWDRGCPS	37.1	1.0	39.6	<0.1 39.6
D820-4-B12-IGFR	RLEQEWAWIQCEVYGRGCRF	35.4	1.0	40.8	<0.1 40.8
	SLEHE*ANVQCKVYGRGC?S	36.2	1.0	41.4	<0.1 41.4

FIG. 6D-2

Clon	Parental/Design	Sequence
D820-4-G9-IGFR	WLDQEWAWVQCEVYGRGCPS	WLDQEWAWVQCEVYGRGCPS
D820-4-C10-IGFR	WLDQEWAWVQCEVWGRGCPS	WLDQEWAWVQCEVWGRGCPS
D820-4-A9-IGFR	WLDLEWFEVQCEVYGRGCPT	WLDLEWFEVQCEVYGRGCPT
D820-4-B8-IGFR	WLEQEWASVQCEVYGRGCPS	WLEQEWASVQCEVYGRGCPS
D820-4-F8-IGFR	WLDLEWEQIKCKVYGRGCPT	WLDLEWEQIKCKVYGRGCPT
D820-4-H7-IGFR	WLEQEWAIQCEVYGRGCPS	WLEQEWAIQCEVYGRGCPS
D820-4-E8-IGFR	WLEQEWALVLCVYGHGCPA	WLEQEWALVLCVYGHGCPA
D820-4-G10-IGFR	WLEQEWAIQCEVWGRGCSS	WLEQEWAIQCEVWGRGCSS
D820-4-D10-IGFR	WLE?EWENWQCEVYGRGC?S	WLE?EWENWQCEVYGRGC?S
D820-4-D8-IGFR	WLEQEWAVQCDVYGRGCPS	WLEQEWAVQCDVYGRGCPS
D820-4-A10-IGFR	WLEQE*ARVQCEVWGRGCPS	WLEQE*ARVQCEVWGRGCPS
D820-4-B7-IGFR	WL?QEWARVHCEVWGRP?QC	WL?QEWARVHCEVWGRP?QC
D820-4-E12-IGFR	PLEHEWAWVQCVYGRGCPS	PLEHEWAWVQCVYGRGCPS
D820-4-H10-IGFR	SLE?EWAWVQCEV?GRGCP?	SLE?EWAWVQCEV?GRGCP?
D820-4-F12-IGFR	WLDQEWVRVQCEVWGRGCPS	WLDQEWVRVQCEVWGRGCPS
D820-4-F7-IGFR	SLDKEWAWKCEVYGRGCPS	SLDKEWAWKCEVYGRGCPS
D820-4-G12-IGFR	LGDQEWAWVEV#GRGWPS	LGDQEWAWVEV#GRGWPS
D820-4-D12-IGFR	WLEEEWAIQRCVYGRGCPS	WLEEEWAIQRCVYGRGCPS
D820-4-A12-IGFR	WLEEE*GWVQCEVWGRGCPP	WLEEE*GWVQCEVWGRGCPP
D820-4-C12-IGFR	CLDQEWAVQCPVYGRGCPS	CLDQEWAVQCPVYGRGCPS
D820-4-A7-IGFR	QLELEWAVQCEVWDRGCPS	QLELEWAVQCEVWDRGCPS
D820-4-B12-IGFR	RLEQEWAWIQCEVYGRGCRF	RLEQEWAWIQCEVYGRGCRF
	SLEHE*ANVQCKVYGRGC?S	SLEHE*ANVQCKVYGRGC?S

Cl ne	Parental/D sign	Sequence
B6-4-G12-IR	WLDQEWAWVQCEVYGRGCPS	WLDQEWAWVQCEVYGRGCPS
B6-3-A11-IR	WLDQEWAWVQCEVYGRGCPS	WLDQEWAWVQCEVYGRGCPS

FIG. 6E

Cl ne Parental/Design	Sequence	Ratios over Background			Comparisons		
		E-Tag 44.8	IGFsR	IR	IGFR/IR	IR/IGFR	
D820-3-D5-IGFR	WLDQEWAWVQCEVYGRGCP	29.6	1.4	24.2	0.1	17.3	
D820-3-E4-IGFR	WVNQALGGVQSDVQRRCS	27.1	3.8	1.0	3.8	0.3	
D820-3-C5-IGFR	LLDHEWPNVGEVGRGSL	25.0	3.2	1.0	3.2	0.3	
D820-3-F4-IGFR	WLHQELAWVRGEGYPRRRS	3.9	3.1	1.0	3.1	0.3	
D820-3-F6-IGFR	WLGHDAWVQCEVYGLGCP	26.7	2.7	1.0	2.7	0.4	
D820-3-G4-IGFR	WIDQEGVRVQCEA*GRAFFS	3.8	2.6	1.0	2.6	0.4	
D820-3-E2-IGFR	WRDEEWAWVQGVQGRGMPA	15.3	2.6	1.0	2.6	0.4	
D820-3-G6-IGFR	RLGVESWVQRRKVGGRDSTS	20.3	2.6	1.0	2.6	0.4	
D820-4-E11-IGFR	WLAQGWAGVQCVYGRGCRN	12.6	2.4	1.0	2.4	0.4	
D820-4-H11-IGFR	WLEEE*AGIQCV?GRGCP	8.1	1.0	3.0	0.3	3.0	
D820-4-D11-IGFR	WLDQEWVQCEVWGRGCLS	4.5	1.0	4.6	0.2	4.6	
D820-4-A8-IGFR	RLQEWALIQCEVYGRGCP	3.2	1.0	5.3	0.2	5.3	
D820-4-F9-IGFR	WLEEEWAQVQCVYGRGCAS	9.4	1.0	5.5	0.2	5.5	
D820-4-C8-IGFR	WLDLE*EWLQCEVYGRGCAT	11.6	1.0	5.8	0.2	5.8	
D820-4-D9-IGFR	WLEQEWVQVRCEVYGRGCP	10.1	1.0	5.9	0.2	5.9	
D820-4-D7-IGFR	WLEEEWAQVQCEVYGRGCP	34.1	1.0	8.9	0.1	8.9	
D820-4-H9-IGFR	WLDQEWARVQCEVWGRGCTY	18.4	3.5	33.4	0.1	9.5	
D820-4-E10-IGFR	YLD?EWAWVQCEVYGLGCS	26.7	1.0	10.1	0.1	10.1	
D820-4-E7-IGFR	WLDVE*AWVQCEVWGRGCP	31.9	2.6	27.0	0.1	10.4	
D820-4-H8-IGFR	WLEQEWER?QCEVYGRGCPP	16.1	3.0	32.2	0.1	10.7	
D820-4-A11-IGFR	WLEEEWAQVQCEVYGRGCLS	8.0	1.0	11.7	0.1	11.7	
D820-4-C9-IGFR	WLDQEWAWIQCEVYGRGCP	19.6	1.0	12.5	0.1	12.5	
D820-4-E9-IGFR	?LEHEWAQIQCEV?GRGCQS	19.3	1.0	14.9	0.1	14.9	
D820-4-B10-IGFR	WL?QEWAWIQCEVYGRGCPF	19.3	1.0	17.3	0.1	17.3	
D820-4-F10-IGFR	WLD?EWAWVQCEVYGRGCP	27.8	1.0	21.5	<0.1	21.5	
D820-4-B9-IGFR	GLEQGCPCWVGLVQCRGCP	31.7	1.0	25.7	<0.1	25.7	
D820-4-G8-IGFR	WLEEEWAWVQCEVYGHGCP	25.6	1.0	26.5	<0.1	26.5	
	WLDQEWAWIQCEVYGRGCSS		1.0	29.3	<0.1	29.3	

FIG. 6D-1

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
D820-3-E5-IR		<u>WLDQEWAWVQCEVYGRGCPS</u>	44.8	1.4	24.2	0.1	17.2
D820-3-D1-IR		WLEQEWITWVQCEVYGCPCPS	25.9	1.0	22.6	<0.1	22.6
D820-3-E1-IR		WLEKEWAGVQCEIYGRGCPS	27.3	1.0	22.4	<0.1	22.4
D820-3-F1-IR		WLEEEWAWVRCEVYGRGCQS	22.4	1.0	21.9	<0.1	21.9
D820-3-B2-IR		WLEHEWAIQCELYGRGCTY	22.0	1.0	21.0	<0.1	21.0
D820-3-A3-IR		ALDEEWAWVQCEVYGRGCPS	13.1	1.0	18.4	0.1	18.4
D820-3-H4-IR		WLEQEWAVQVQCEVYGRGCPS	23.5	1.0	18.4	0.1	18.4
D820-3-G1-IR		WLDDEWAIQCEIYGRGCQS	25.6	1.0	17.5	0.1	17.5
D820-3-C1-IR		QLEEEWAGVQCEVYGRGCPS	14.5	1.0	16.3	0.1	16.3
D820-3-A1-IR		WLEQEWLLVQCGVYGRGCPS	27.8	1.0	13.9	0.1	13.9
D820-3-A5-IR		WLDQEWAWIQCEVYGRGCPS	14.7	1.0	12.8	0.1	12.8
D820-3-H1-IR		WLEQEWAVQVQCEVSGRGCP	6.4	1.0	6.3	0.2	6.3
D820-3-A4-IR		W?DQEWALIQCEVYGRGCPS	13.7	1.0	6.2	0.2	6.2
D820-4-E12-IR		SLDEEWAGVLCVYGRGCPF	6.0	1.0	4.3	0.2	4.3
D820-4-B12-IR		SVDQELEWLMCHFQGRVCP	34.9	9.0	10.9	0.8	1.2
		WLEQERAWIWCEIQGSGCRA	32.2	8.6	1.0	8.6	0.1

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FIG. 6C-2

Clone	Parental/Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
D820-3-H2-IR		<u>WLDQEWAWVQCEVYGRGCP</u> S	44.8	1.4	24.2	0.1 17.2
D820-3-H2-IR		RLDLEWANIQCEVYGRGCP	23.9	1.0	40.0	<0.1 40.0
D820-3-C4-IR		WLEQEWARVQCEVYGRGC	31.0	1.0	39.5	<0.1 39.5
D820-3-C3-IR		WLEQEWILVECEVYGRGCP	35.2	1.0	39.4	<0.1 39.4
D820-3-G6-IR		WLEQEWAVQCEVWGRGCP	33.8	1.0	38.8	<0.1 38.8
D820-3-D2-IR		WLDQEWEWIQCEVYGRGCP	35.6	1.0	37.8	<0.1 37.8
D820-3-D3-IR		LLDEEWAQIECEIYGRGCP	34.8	1.0	37.7	<0.1 37.7
D820-3-B5-IR		ALDEEWAWVQCEVYGRGCH	34.1	1.0	37.1	<0.1 37.1
D820-3-E2-IR		C?EQEWGLVQCEVYGRGCP	34.4	1.0	37.0	<0.1 37.0
D820-3-B3-IR		WLEQEWAYVQCEVYGRGCP	33.6	1.0	36.7	<0.1 36.7
D820-3-B6-IR		WLEHEWAQVQCEVWGRGCP	31.2	1.0	36.6	<0.1 36.6
D820-3-D4-IR		WLEQEWAEVRCVYGRGCP	32.0	1.0	36.2	<0.1 36.2
D820-3-C2-IR		?LEQEWAWVQCEVYGRGCP	33.7	1.0	35.6	<0.1 35.6
D820-3-F6-IR		WLEQEWAGIQCKVYGRGCP	30.8	1.0	35.2	<0.1 35.2
D820-3-D5-IR		RLEQEWAVQCEVWGRGCLP	30.5	1.0	34.8	<0.1 34.8
D820-3-F5-IR		QLDHEWAGIQCEVWGRGCP	29.8	1.0	34.6	<0.1 34.6
D820-3-H3-IR		WLEQEWAGIQCEVYAGCR	30.2	1.0	33.8	<0.1 33.8
D820-3-G2-IR		SLEQEWAWVQCVYGRGCP	31.3	1.0	33.0	<0.1 33.0
D820-3-H6-IR		WLEQEWQVLCVYGRGCP	30.3	1.0	32.2	<0.1 32.2
D820-3-F3-IR		WLEQEWAV?CEVYGRGCA?	28.6	1.0	30.7	<0.1 30.7
D820-3-B4-IR		WMDQEWAWVQCEVYGRGCP	33.1	1.0	30.5	<0.1 30.5
D820-3-C5-IR		QLDQEWAWIQCEVYGRNCRT	29.1	1.0	30.3	<0.1 30.3
D820-3-F4-IR		TLEQEWAVQVCEVYGRGCLS	25.9	1.0	29.5	<0.1 29.5
D820-3-H5-IR		RLEQEWAVQCEVWGRGCLS	26.3	1.0	28.6	<0.1 28.6
D820-3-A6-IR		WLDQEWALVQCEVYGRGCPA	24.8	1.0	26.0	<0.1 26.0
D820-3-A2-IR		WLDQEWAVQCHVWGRGCPA	23.7	1.0	25.6	<0.1 25.6
D820-3-G5-IR		WLEQEWAWVQCEVYGRGCP	22.6	1.0	25.0	<0.1 25.0
D820-3-G3-IR		RLEEWAWVQCVYGRGCP	22.2	1.0	23.9	<0.1 23.9
D820-3-E3-IR		WLEQEWVRIQCEVYGRGCP	20.6	1.0	22.7	<0.1 22.7

FIG. 6C-1

Clone	Parental/Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
D815-4-G8-IR		<u>WLDQEWAWVQCEVYGRGCPS</u>	--	--	--	--
D815-4-G7-IR		<u>QLDQEWARVRCEVWGRGCSS</u>	27.8	1.0	33.6	<0.1 33.6
D815-4-G11-IR		<u>WLDLEWAQVQCKVYGRGCPS</u>	34.7	1.0	32.3	<0.1 32.3
D815-4-E7-IR		<u>WLDEEWAWVQCQVYGRGCPS</u>	30.7	1.0	28.6	<0.1 28.6
D815-4-A12-IR		<u>WLDQEWAWVQCEVWGRGCAF</u>	33.0	1.0	26.4	<0.1 26.4
D815-4-B11-IR		<u>WLDREWAQVQCEVYGRGCLS</u>	28.4	1.0	19.0	0.1 19.0
D815-4-D8-IR		<u>WLDAEWVQCEVYGRGCRP</u>	22.1	1.0	18.8	0.1 18.8
		<u>SLDREWAYVQCQVYGRGCSS</u>	20.8	1.0	14.6	0.1 14.6

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FIG. 6B-2

Clone		Parental/Design		Sequence		Ratios over Background		Comparis ns	
E-Tag		IGFsR		IR		IGFR/IR		IR/IGFR	
44.8	1.4	24.2	17.3	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	44.8	1.4	24.2	17.3
48.0	1.0	48.4	48.4	WLDLEWAQVQCEVYGRGCP	WLDLEWAQVQCEVYGRGCP	48.0	1.0	48.4	48.4
48.2	1.0	48.2	48.2	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	48.2	1.0	48.2	48.2
47.5	1.0	48.0	48.0	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.5	1.0	48.0	48.0
47.9	1.0	48.0	48.0	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.9	1.0	48.0	48.0
49.0	1.0	47.6	47.6	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	49.0	1.0	47.6	47.6
45.4	1.0	47.2	47.2	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	45.4	1.0	47.2	47.2
49.5	1.0	47.0	47.0	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	49.5	1.0	47.0	47.0
48.1	1.0	46.6	46.6	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	48.1	1.0	46.6	46.6
47.8	1.0	46.4	46.4	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.8	1.0	46.4	46.4
47.7	1.0	45.8	45.8	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.7	1.0	45.8	45.8
47.8	1.0	45.8	45.8	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.8	1.0	45.8	45.8
49.0	1.0	45.6	45.6	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	49.0	1.0	45.6	45.6
49.0	1.0	45.6	45.6	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	49.0	1.0	45.6	45.6
47.0	1.0	45.6	45.6	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.0	1.0	45.6	45.6
44.5	1.0	45.4	45.4	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	44.5	1.0	45.4	45.4
44.2	1.0	44.2	44.2	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	44.2	1.0	44.2	44.2
44.3	1.0	43.7	43.7	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	44.3	1.0	43.7	43.7
45.5	1.0	43.0	43.0	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	45.5	1.0	43.0	43.0
46.2	1.0	43.0	43.0	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	46.2	1.0	43.0	43.0
47.2	1.0	42.6	42.6	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.2	1.0	42.6	42.6
47.9	1.0	42.6	42.6	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.9	1.0	42.6	42.6
46.4	1.0	41.8	41.8	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	46.4	1.0	41.8	41.8
47.3	1.0	41.2	41.2	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.3	1.0	41.2	41.2
37.7	1.0	40.0	40.0	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	37.7	1.0	40.0	40.0
47.0	1.0	39.8	39.8	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	47.0	1.0	39.8	39.8
44.2	1.0	39.8	39.8	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	44.2	1.0	39.8	39.8
40.4	1.0	39.2	39.2	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	40.4	1.0	39.2	39.2
45.4	1.0	38.6	38.6	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	45.4	1.0	38.6	38.6
37.3	1.0	37.3	37.3	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	37.3	1.0	37.3	37.3
2.4	1.0	37.2	37.2	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	2.4	1.0	37.2	37.2
32.4	1.0	34.7	34.7	WLDQEWAWVQCEVYGRGCP	WLDQEWAWVQCEVYGRGCP	32.4	1.0	34.7	34.7

FIG. 6B-1

Ratios over Background		Comparisons	
E-Tag	IGFsR	IGFR/IR	IR/IGFR
--	--	--	--
50.2	1.6	0.1	14.4
44.2	1.3	0.1	18.5
44.8	1.4	0.1	17.3

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Sequence
 XXXXXXXXXXXXXXXXXXXXXXXX
 EIEAEWGRVRCCLVYGRGVGG
 EIEAEWGRVRCCLVYGRGVGG
 WLDQEWAWVQCEVYGRGCPS

Clone
 Design
 R20α-3-20A4-IR
 R20β-4-A7-IR
 R20β-4-D8-IR

FIG. 6A

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Clon Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
20C-3-A1-IR	HLCVLEELFWGASLFGYCSCG	--	--	--	--
20C-3-C1-IR	RLCALEELFWGASLFGQCSCG	21.0	1.1	17.6	0.1
20C-3-D2-IR	HLCVLEELFWGALFHQCSCG	30.6	1.4	21.9	0.1
B6C-4-G12-IR	RLCVLEELFWGASLFGQCSCG	7.0	1.1	14.9	0.1
B6H-4-F9-IR	QLCVLEELFWGSSRLGYCSCG	31.1	2.5	33.5	0.1
B6C-4-E3-IR	DLCVLEELFWGASLFGQCSCG	39.3	3.6	43.1	0.1
20C-3-B10-IR	QLCLLEELFWGSSLFGQCSCG	34.6	5.3	40.0	0.1
20C-3-A3-IR	HLCVLEELFWGTSLFGQCSCG	29.9	16.9	31.7	0.5
	RLCVLEELFWGASLFDQCSCR	28.4	19.1	25.3	0.8
					1.3

FIG. 4E-3

Clon Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
20C-3-A1-IR	HLCVLEELFWGASLFGYCSCG	39.1	1.8	27.7	0.1
20C-3-C1-IR	PLQALCEKFFGAWMEGYCSCG	31.2	13.9	1.0	13.9
20C-3-D2-IR	HLQVLCELFGGVYLFYCYCSCG	27.2	19.2	1.7	11.3
B6C-4-G12-IR	PLFDLCELFGGASLSGYCYG	35.4	17.4	1.6	10.9
B6H-4-F9-IR	HL*ALCELFGGVWSFGYCVG	29.5	16.9	1.7	9.9
B6C-4-E3-IR	QLGVLCMFAGFRLLGYCQG	36.6	25.7	2.7	9.5
20C-3-B10-IR	HLQDLCELFGGAYLFGYCSCG	29.6	16.0	3.8	0.2
20C-3-A3-IR	QLQVLCELFGGAVSLRLLW	33.7	3.5	1.0	3.5
	PLGVLCFQFGGAFRFGYCSCG	33.6	18.9	9.9	1.9
	PL*GLCELFGGASLFGYCSC	7.5	1.7	2.3	0.7
	DLRVLCELFGGAYVLGYCSE	35.1	3.7	12.6	0.3
					3.4

FIG. 5

Clon Design	Sequence
20C-3-A1-IR	HLCVLEELFWGASLFGYCSCG
20C-3-C1-IR	RLCALEELFWGASLFGQCSCG
20C-3-D2-IR	HLCVLEELFWGALFHQCSCG
B6C-4-G12-IR	RLCVLEELFWGASLFGQCSCG
B6H-4-F9-IR	QLCVLEELFWGSSRLGYCSCG
B6C-4-E3-IR	DLCVLEELFWGASLFGQCSCG
20C-3-B10-IR	QLCLLEELFWGSSLFGQCSCG
20C-3-A3-IR	HLCVLEELFWGTSLFGQCSCG
	RLCVLEELFWGASLFDQCSCR

Clon Parental/Design	Sequence
F815-4-D10-IGFR	HLCVLEELFWGASLFGYCSCG
F815-4-H11-IGFR	PLQALCEKFFGAWMEGYCSCG
F815-4-C8-IGFR	HLQVLCELFGGVYLFYCYCSCG
F815-4-E8-IGFR	PLFDLCELFGGASLSGYCYG
F815-4-E11-IGFR	HL*ALCELFGGVWSFGYCVG
F815-4-A7-IGFR	QLGVLCMFAGFRLLGYCQG
F815-3-D3-IGFR	HLQDLCELFGGAYLFGYCSCG
F815-4-F7-IGFR	QLQVLCELFGGAVSLRLLW
F815-4-A9-IGFR	PLGVLCFQFGGAFRFGYCSCG
F815-4-B12-IGFR	PL*GLCELFGGASLFGYCSC
	DLRVLCELFGGAYVLGYCSE

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
20C-3-B4-IR	HLCVLEELFWGASLFGYCSCG	28.9	1.1	31.1	--
20C-3-C11-IR	NLCVLEELFWGESLFGQCSG	30.2	1.1	31.0	<0.1
B6C-4-G2-IR	HLCVLEEQFWGCSLFGYCSCG	29.4	1.3	35.3	<0.1
20C-3-B8-IR	HLCFLEEVFWGAALFAQCSG	28.5	1.1	31.2	<0.1
20C-3-C10-IR	HLCDLEVLFWGSALFGQCSG	32.1	1.2	33.6	<0.1
20C-3-B6-IR	HLCVMEELFWGASLFGQCSG	29.7	1.2	31.9	<0.1
A6L-3-A3-IR	HLCVLEEQWGESLFGYCSCG	14.4	1.1	28.3	<0.1
A6L-3-B3-IR	PLCVLEEQFWGASLFAYCSCG	38.7	1.7	43.4	<0.1
20C-3-A5-IR	QLCVLEELFWGESLFAQCLG	22.9	1.1	27.6	<0.1
20C-3-B11-IR	HLCVLEELFWQSLFGHCSD	30.0	1.3	32.7	<0.1
20C-3-B3-IR	HLCVLEELVWGASLFGFCSCG	29.3	1.2	31.2	<0.1
20C-3-C12-IR	LLCVLEEQFWGASLFGQCSG	29.6	1.3	31.8	<0.1
20C-3-C3-IR	RLCVLEELFWGESLFGQCSG	30.1	1.2	30.1	<0.1
20C-3-C2-IR	HLCVLEEMFWGASLFGNCSCG	29.9	1.3	29.8	<0.1
20C-3-A11-IR	ELCFLEELFWGASLFGQCSG	25.9	1.2	27.4	<0.1
20C-3-A4-IR	HLCVLEELFWGASLYGQCSS	27.2	1.2	27.5	<0.1
20C-3-A6-IR	HLCVLEELFWGASLFAQCPG	26.1	1.2	27.5	<0.1
B6C-4-E4-IR	NLCVLEELFWGASEFGQCSG	34.5	1.7	39.1	<0.1
20C-3-A9-IR	DLCVLEEQWGLWASLFGYCSCG	29.7	1.3	29.3	<0.1
B6C-3-C5-IR	HLCVLEEQFWGVALFGNCSCG	33.5	1.7	37.7	<0.1
20C-3-B1-IR	HLCVLEEQWGLWASLFGQCSG	30.2	1.2	26.7	<0.1
20C-3-A10-IR	HLCVLEERFWGGALFGQCTA	29.0	1.3	28.5	<0.1
20C-4-F1-IR	HLCDLEELFWGASLFGQCSG	29.1	1.4	29.5	<0.1
20C-4-E1-IR	QLCVLEELFWGTSLFGAGCSG	28.3	1.4	29.7	<0.1
20C-3-B12-IR	QLCGLEELFWGASLFGYCSCG	27.0	1.3	25.8	<0.1
20C-3-A8-IR	HLCVLEELFWGASLFGQCSS	21.1	1.1	21.2	0.1
20C-3-A7-IR	FLCVLEELYWGASQFGQCSG	21.9	1.3	23.0	0.1
B6C-4-E10-IR	HLCVLEEQFWGASLFGYCSCG	35.2	2.2	38.0	0.1

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FIG. 4E-2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
A6L-3-C4-IR	HLCVLEELFWGASLFGQCSG	36.9	1.0	--	--
A6L-3-D7-IR	DLCVLEERFWGASLFGQCSG	38.6	1.0	<0.1	42.5
A6L-3-A1-IR	QLCVLEELHWGASLFGQCSG	39.6	1.1	<0.1	40.7
A6L-3-C1-IR	PLCVLEEQFWGASLFGQCSG	37.3	1.0	<0.1	40.6
A6L-3-D5-IR	YLCDLEERFWGASLFGQCSG	42.9	1.1	<0.1	40.3
A6L-3-A4-IR	HLCLEERFWGSSQFGFCG	26.7	1.1	<0.1	40.2
A6L-3-D3-IR	HLCVLEELFWGASQFGQCSG	34.6	0.9	<0.1	39.8
A6L-3-B1-IR	HLCVLEERFWGASLFGQCSG	33.9	1.0	<0.1	39.3
A6L-3-B5-IR	HLCVMEELFWGTSLFGQCTG	35.3	1.1	<0.1	38.6
A6L-3-B2-IR	HLCVLEERFWGASLFGQCSG	38.1	1.1	<0.1	37.7
B6H-4-G12-IR	HLCVLEELFWGASLFGQCSG	31.6	1.1	<0.1	36.7
B6C-4-H10-IR	QLCVLEELFWGASLFGQCSG	38.5	1.1	<0.1	36.5
B6H-4-G8-IR	HLCVLEEMFWGASLFGQCSG	31.7	1.1	<0.1	36.2
A6L-3-D6-IR	HLCVLEELFWGASLFGQCSG	35.5	1.0	<0.1	36.1
B6C-4-F1-IR	QLCVLEELFWGASQFGYCSG	32.9	1.1	<0.1	35.8
B6C-4-H3-IR	QLCALEQFWGASLFGQCSG	37.4	1.2	<0.1	34.8
B6H-4-E8-IR	QLCVLEELFWGASLFGQCSG	30.2	1.0	<0.1	34.3
B6C-4-G1-IR	HLCVLEEFWGDLSLFGQCSR	34.9	1.2	<0.1	33.7
B6H-4-E9-IR	HLCVLEERFWGASLFGQCSG	34.4	1.2	<0.1	33.2
B6C-4-F5-IR	QLCELEEVFWGASLFDYCSG	34.7	1.2	<0.1	32.8
B6C-4-F11-IR	HLCVLEELFWGASRFGQCSG	34.0	1.2	<0.1	31.7
B6C-4-E6-IR	HLCVLEELFWGASLFGQCSA	32.3	1.2	<0.1	30.6
B6C-4-E12-IR	HLCVLEELIWGASRFGQCSG	30.9	1.1	<0.1	30.2
B6C-4-G10-IR	HLCVLEELFWGGSFLFIQCSG	33.0	1.3	<0.1	30.1
B6C-4-F8-IR	QLCVLEEQFWGASLFGNCSG	36.4	1.4	<0.1	29.3
20C-3-B5-IR	HLCVLEERFWGAALFGQCSG	26.6	1.1	<0.1	29.2
B6C-4-G3-IR	HLCVLEEMFWGASLFGQCGG	34.0	1.4	<0.1	28.3
20C-3-B7-IR	PLCVLEELFWGASLFGQCSG	29.5	1.2	<0.1	28.3

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FIG. 4E-1

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Cl ne Parental/design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGFsR --	IGFR/IR --	IR/IGFR --
F820-4-A8-IR	HLCVLEELFWGASLFGYCSCG	6.4	0.7	2.4	0.3
F820-4-G1-IR	QLCVMEELFWGASRFQCSCG	3.9	0.6	1.9	0.3
F820-4-F3-IR	HLCVLEELFWGASMFQCSCG	9.8	1.3	3.6	0.4
F820-4-D6-IR	QLCVLEEMFWGGSRFVQCSA	5.4	1.2	3.2	0.4
F820-4-A1-IR	PLCVLEELFWGEALFDQCGA	25.5	2.4	6.1	0.4
F820-4-H2-IR	YLCVQEEELFWGASLFGYCSV	15.9	1.6	4.1	0.4
F820-4-F4-IR	HLCVLEERFWGASLFGQCSG	6.8	1.9	4.7	0.4
F820-4-B6-IR	QLCDLEELFWGASLFGYCPG	4.1	0.8	1.9	0.4
F820-4-B11-IR	HLCVLEERFWGASLFGYCPG	22.2	3.1	7.0	0.4
F820-4-H6-IR	QLCVLEELFWGASLFGYCPG	4.1	1.1	2.4	0.5
F820-4-H9-IR	PLCVLEELFWGAAQFGQCSG	3.1	0.9	1.9	0.5
F820-4-D3-IR	QLCDLEERFWGVSFLGLCSG	4.6	1.3	2.5	0.5
F820-4-C1-IR	QLCVLEEVFWGASLFGGLCTG	13.0	1.1	2.1	0.5
F820-4-D12-IR	QLDLNTWSGLCLCSVTTRV	10.4	1.2	2.0	0.6
F820-4-B8-IR	DLCVLEESLWGKALFGYCSD	7.2	2.2	3.4	0.6
F820-4-C6-IR	HLCVLEEVFWGSSMFGDCSG	13.9	2.5	2.8	0.9
F820-4-C10-IR	HLCVLEELFWGASLFGDCQG	5.3	2.6	2.9	0.9
F820-4-D4-IR	QLCVLDALMWGGCRLGHQCG	3.5	2.3	2.1	1.1
F820-4-E1-IR	QLCVLEEKFWGTSLFGDCMG	1.6	1.6	1.5	1.1
F820-4-B3-IR	HLCVLEEVFWGAAQFGSCSG	15.9	0.6	5.0	1.2
F820-4-D2-IR	QLCVLEELFWGSPMFGYCSCG	7.8	3.2	2.5	1.3
F820-4-C5-IR	HLCVLEELFWGASGFAQCYG	21.5	4.0	2.3	1.8

FIG. 4D-2

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Ratios over Background				Comparisons	
E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
39.1	1.8	27.7	0.1	15.4	
28.1	0.9	17.9	<0.1	21.1	
34.0	1.6	22.7	0.1	13.9	
21.3	0.7	9.0	0.1	13.4	
34.1	1.0	12.1	0.1	12.1	
14.2	0.6	6.5	0.1	11.6	
14.0	0.5	6.1	0.1	11.5	
38.1	1.2	11.8	0.1	9.9	
15.1	0.7	6.4	0.1	8.7	
46.3	2.7	22.2	0.1	8.2	
14.5	0.6	4.7	0.1	8.0	
8.8	0.6	4.4	0.1	7.5	
11.7	0.6	4.2	0.1	7.4	
16.0	0.6	4.7	0.1	7.3	
14.5	0.8	5.9	0.1	7.1	
5.0	0.4	2.4	0.2	6.9	
37.5	1.1	7.5	0.2	6.6	
21.2	1.1	6.4	0.2	5.9	
7.5	0.7	3.7	0.2	5.6	
5.3	0.8	4.4	0.2	5.2	
5.7	0.6	3.0	0.2	4.9	
9.1	1.0	4.7	0.2	4.7	
5.9	0.8	3.5	0.2	4.5	
25.7	2.3	10.5	0.2	4.5	
3.7	0.6	2.7	0.2	4.2	
11.3	0.6	2.2	0.3	3.5	
7.6	0.8	2.7	0.3	3.5	
8.0	1.2	4.3	0.3	3.5	
17.5	2.6	9.0	0.3	3.4	

FIG. 4D-1

Clone	Parental/Design	Sequence	Ratios over Background				Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
F815-4-F11-IGFR		HLCVLEELFWGASLFGYC SG	39.1	1.8	27.7	0.1	15.4	
F815-4-E12-IGFR		PLCFLOELFGGASLGGYC SG	33.4	12.3	1.0	12.3	0.1	
F815-4-H10-IGFR		FMCGLOELVGGAA LLGHCSG	33.7	15.1	1.7	8.9	0.1	
F815-4-B7-IGFR		PLCFLOELFGGSLSGYC SG	30.1	8.5	1.0	8.5	0.1	
F815-3-B5-IGFR		FLCGLEELANGVSRSGYC FG	35.2	23.9	4.8	5.0	0.2	
F815-4-D12-IGFR		PLCFIAELFSGSALGGDC SR	33.9	4.8	1.0	4.8	0.2	
F815-4-C11-IGFR		PLCVLOELFGGSLGGYC SG	33.6	7.0	1.8	3.9	0.3	
F815-4-C7-IGFR		QLCVLE#LFWACLFGYC AG	13.9	4.6	1.8	2.6	0.4	
F815-4-E7-IGFR		FLCGLOELSGVASLFGQ CSG	16.8	2.0	1.0	2.0	0.5	
F815-4-G7-IGFR		RVCVLEQLVWGASLFGA* SG	26.9	3.8	1.9	2.0	0.5	
F815-4-A10-IGFR		FYCGLEELSWGALFGYC SG	30.4	9.0	5.0	1.8	0.6	
F815-3-B3-IGFR		FLCGLEELSQQGAVLFGH CYG	30.8	3.7	2.2	1.7	0.6	
F815-3-G1-IGFR		HLCVLVGLFWDASLFGQ CSG	7.6	1.0	2.0	0.5	2.0	
F815-4-G12-IGFR		QRCIRAAALFWCATLLGGC AG	20.5	1.0	2.0	0.5	2.0	
F815-3-H1-IGFR		HQCIPDGMSQGAALRGNC SD	7.6	1.0	2.5	0.4	2.5	
		HLCVLEDELNGVSLFGYC SS	18.4	1.0	6.8	0.1	6.8	

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FIG. 4C

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Clone	D sign	Sequence	Ratios over Background		Comparisons		
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
F815-4-F11-IR		<u>HLCVLEELFWGASLFGYCSCG</u>	31.8	1.0	33.7	<0.1	34.2
F815-3-A9-IR		RLCVLEERFWGAALFGQCSG	31.9	1.0	35.5	<0.1	34.1
F815-4-G11-IR		PLCVLEELFWGASLFGQCSG	32.3	1.0	34.4	<0.1	33.9
F815-3-D8-IR		SLCVLEELFWGSRFGQCSG	32.3	1.0	33.3	<0.1	33.7
F815-4-G4-IR		HLCVLEEQFWGASLFGYCFC	23.8	1.0	32.2	<0.1	33.7
F815-3-C8-IR		HLCVLEEQFWGASLFGQCSG	33.9	1.0	35.1	<0.1	33.6
F815-4-G12-IR		DLCLLEELLWGASRFGQCSG	31.7	1.0	33.5	<0.1	33.5
F815-3-D12-IR		YLCVLEERFWGASLFGQCSG	33.3	1.0	34.8	<0.1	33.4
F815-4-F7-IR		HLCVLEEQFWGASLFGSCSG	33.3	1.0	34.3	<0.1	33.4
F815-4-F2-IR		QLCVLEELQWGASLFGQCSG	26.1	1.0	33.8	<0.1	33.3
F815-3-B9-IR		HLCVLEELF*GESLFGYCSCG	33.6	1.1	35.7	<0.1	33.2
F815-4-H2-IR		HLCVLEELFWGASLFGQCSG	36.1	1.2	38.4	<0.1	33.0
F815-4-E11-IR		PLCVLEELFWGASHFGQCSG	33.2	1.1	35.4	<0.1	33.0
F815-4-G1-IR		HLCVLEELVWGASLFGQCSG	27.9	1.0	31.5	<0.1	32.8
F815-3-A11-IR		QLCVLEELVWGASLFGQCSG	37.7	1.2	40.1	<0.1	32.7
F815-4-F6-IR		HLCVLEELFWGASLFGQCSG	32.3	1.1	34.6	<0.1	32.6
F815-3-D9-IR		HLCVLEELVWGASLFGQCSG	31.4	1.0	32.5	<0.1	32.5
F815-3-C11-IR		RLCVLEELFWGASLFGQCSG	33.4	1.1	35.7	<0.1	31.9
F815-4-G2-IR		HLCVLEELFWGATLFDQCSG	30.2	1.1	34.3	<0.1	31.4
F815-3-C9-IR		HLCVLEELFWGASLFGQCSG	29.7	1.0	31.4	<0.1	31.0
F815-4-H10-IR		HLCVLEELFWAAPLFGQCSG	31.9	0.9	27.6	<0.1	29.4
F815-4-F3-IR		HLCVLEELVWGASLFGQCSA	19.4	1.0	28.0	<0.1	28.9
F815-4-F5-IR		NLCVLEELFWGASQFRYCPG	12.3	0.9	24.8	<0.1	26.8
F815-4-H1-IR		RLCVLEELFWGASLFGQCSG	6.9	1.0	15.8	0.1	16.5
F815-4-E5-IR		PLCVLEELFWGASLFGQCPG	3.5	1.0	13.6	0.1	14.0
F815-4-H5-IR		NLCVLEELFWGASLFGQCSG	5.5	1.0	13.1	0.1	13.5
F815-3-C10-IR		OLCVLG#RFGGSLCGYCS	3.5	1.1	5.2	0.2	4.5

FIG. 4B-3

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
F815-3-A6-IR	HLCVLEELFWGASLFGYCSCG	34.6	1.1	39.0	<0.1	36.2	36.2
F815-3-D3-IR	QLCVLEELFWGSSSLFGQCSCG	33.8	1.0	36.2	<0.1	36.2	36.2
F815-3-B12-IR	DLCVVEELFWGKSLFGQCSCG	33.2	1.0	35.7	<0.1	36.2	36.2
F815-4-G10-IR	DLCVLEELFWGSSSLFGQCSCG	35.4	1.0	37.2	<0.1	36.1	36.1
F815-4-E3-IR	YLCVLEEQFWGASLFRQCFCG	32.4	1.0	35.0	<0.1	36.1	36.1
F815-4-E6-IR	HLCVLEELLWGSSSLFGQCSCG	33.2	1.0	34.5	<0.1	36.1	36.1
F815-4-F1-IR	PLCGLEELFWGASLFGQCSD	29.4	0.9	32.5	<0.1	36.0	36.0
F815-4-G8-IR	HLCVLEELFWGSSSLFAQCSCG	36.8	1.1	38.2	<0.1	35.9	35.9
F815-4-H12-IR	PLCAIEELFWGAALFGQCSCG	30.5	0.9	31.9	<0.1	35.9	35.9
F815-4-G3-IR	HLCVLEEQFWGASLFGDCSCG	31.4	1.0	35.7	<0.1	35.7	35.7
F815-3-C2-IR	PLCVLEELFWGAPLFGQCSD	32.3	1.0	36.1	<0.1	35.6	35.6
F815-4-E10-IR	DLCGLEELFWGAALFGQCTS	35.4	1.0	36.5	<0.1	35.4	35.4
F815-3-A12-IR	QLCVLEKQLWGASLFWQCSCG	32.1	1.0	36.3	<0.1	35.3	35.3
F815-3-B8-IR	HLCVLEELFWGASLYQCPCG	33.6	1.0	35.8	<0.1	35.3	35.3
F815-3-B2-IR	HLCVLEELFWGASLFDQCSCG	31.0	1.0	35.3	<0.1	35.3	35.3
F815-3-C3-IR	HLCVLEELLWGASLFGQCSCG	30.1	1.0	35.3	<0.1	35.3	35.3
F815-3-A7-IR	PLCVLEELFWGVSLFGQCGG	33.1	1.0	35.8	<0.1	35.2	35.2
F815-4-F9-IR	HLCVLEELFWGASQWQCSCG	33.4	1.0	35.7	<0.1	35.2	35.2
F815-3-B7-IR	RLCVLEEQFWGGALFGQCSCG	32.0	1.0	33.5	<0.1	35.0	35.0
F815-4-E4-IR	QLCVLEELFWGVSLFAQCSCG	28.0	1.0	33.4	<0.1	35.0	35.0
F815-4-E12-IR	HLCVLEELFWGAALFGQCFCG	28.0	0.9	30.2	<0.1	34.8	34.8
F815-4-F8-IR	YLCVLEELFWGASQFGQCSCG	33.8	1.0	35.2	<0.1	34.7	34.7
F815-3-C7-IR	HLCVLEELYWGASLFGQCSCG	33.9	1.0	34.7	<0.1	34.7	34.7
F815-4-F10-IR	HLCVLEERFWGVSLFGQCSCG	32.7	1.0	34.2	<0.1	34.7	34.7
F815-3-D11-IR	PLCVLEELFWGASRFGQCSCG	35.4	1.1	37.3	<0.1	34.6	34.6
F815-4-E7-IR	HLCVLEDLFWGASLFDQCSCG	30.3	0.9	32.2	<0.1	34.6	34.6
F815-3-A10-IR	HLCVLELVFWGASLFGQCSCG	34.0	1.1	36.4	<0.1	34.3	34.3
F815-3-B11-IR	QLCIIEEWFNGTSLFGYCSCG	33.7	1.1	36.3	<0.1	34.2	34.2
	ALCVLEELFWGESLFGQCSCG						

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FIG. 4B-2

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
F815-4-H9-IR	<u>HLCVLEELFWGASLFGYC</u> SG	--	--	--	--
F815-3-B1-IR	PLCVLEELFWSTPLFGQCSY	34.9	0.9	<0.1	40.8
F815-3-D1-IR	HLCVLEELFWGASLFAQCVG	31.7	0.9	<0.1	39.3
F815-3-D1-IR	DLCVLEELFWGASRFGQCSG	30.4	0.9	<0.1	38.9
F815-3-D4-IR	HLCVLEELFWGASLFGQCSG	31.5	0.9	<0.1	38.8
F815-3-C5-IR	HLCVVEELFWGASLFGQCSG	31.1	0.8	<0.1	38.5
F815-4-H3-IR	NLCVLEELFWGASLFRQCSG	33.7	1.0	<0.1	38.4
F815-3-A5-IR	PLCVLEELFWGASLFGQCSG	37.4	1.1	<0.1	38.3
F815-3-D7-IR	QLCVLEELFWGASEFGQCSG	33.6	0.9	<0.1	38.3
F815-3-A1-IR	HLCVLEELFWGASLFGQCSG	29.8	0.9	<0.1	38.0
F815-4-H4-IR	PLCVLEELFWGESLFGQCSG	31.1	0.9	<0.1	38.0
F815-3-A3-IR	HLCVLEELFWGASRFGQCSG	32.8	1.0	<0.1	37.9
F815-3-B3-IR	KLCVLEELFWGASLFGQCSG	33.7	1.0	<0.1	37.5
F815-3-A4-IR	YLCVLEELSWGASLFGQCSG	32.5	1.0	<0.1	37.5
F815-3-D2-IR	HLCVLEELWASLFAQCVG	31.9	0.9	<0.1	37.4
F815-3-C4-IR	QLCVLEELFWGESLFGQCSG	31.6	0.8	<0.1	37.4
F815-3-B4-IR	HLCVLEELFWGNLFSQCSG	33.8	1.0	<0.1	37.3
F815-3-C1-IR	HLCVLEELFWGASLYGQCSG	29.0	0.9	<0.1	37.3
F815-4-G9-IR	SLCALEEQFWGAALFGYCSG	36.5	1.0	<0.1	37.1
F815-4-G6-IR	HLCVLEELFWGASLFDGCAG	34.9	1.0	<0.1	37.0
F815-3-A8-IR	QLCVLEELFWGASLFGQCSG	34.7	1.1	<0.1	36.9
F815-4-G5-IR	PLCVLEELFWGAALFGQCSG	26.5	1.0	<0.1	36.8
F815-3-B5-IR	HLCVLEELFWGASLFGQCTG	33.2	0.9	<0.1	36.8
F815-4-F4-IR	PLCVLEELFWGGSFLFGQCSG	28.6	0.8	<0.1	36.7
F815-3-A2-IR	QLCVLEELWASLFGQCSG	32.5	1.0	<0.1	36.6
F815-3-B6-IR	HLCVVEELWASLFGQCSR	31.6	0.9	<0.1	36.5
F815-4-H7-IR	DLCVLEELFWGASLFGQCSG	33.7	1.0	<0.1	36.4
F815-4-H8-IR	QLCVLEERFWGASLFGQCSG	35.8	1.0	<0.1	36.4
F815-4-G7-IR	NLCVLEELFWGAALFGQCSG	33.7	1.0	<0.1	36.3

FIG. 4B-1

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
rB6-3-F1-IGFR	XXXLXXLXXYFXXXXXXXX	26.5	5.8	1.4	4.1	0.2
rB6-4-B7-IGFR	NTILGDLWRYFAGSGGM	31.1	11.4	2.9	3.9	0.3
rB6-4-C10-IGFR	?DVLKKL?VYFELSGGA	32.2	3.7	1.0	3.7	0.3
rB6-3-A1-IGFR	GGPLQGLYTYFKQSPVC	21.1	3.5	1.0	3.5	0.3
rB6-3-F6-IGFR	DRLLSGLWAYFAGNGGS	25.1	3.5	1.0	3.5	0.3
rB6-3-H5-IGFR	DLILQSLLDYFQGRPVG	35.5	18.1	5.6	3.2	0.3
rB6-4-D8-IGFR	LALLPMLWDYFVATDPQ	36.2	22.5	7.5	3.0	0.3
rB6-4-A8-IGFR	DSILRELRDYFARTHIA	41.1	30.6	10.6	2.9	0.3
rB6-4-H8-IGFR	DGVLGQLWQYFAQYPGS	38.7	33.0	11.5	2.9	0.3
rB6-3-E2-IGFR	?PPLDALWEYFTGTARD	20.9	2.2	1.0	2.2	0.5
rB6-3-C2-IGFR	DNVLEGLWSYFALWSQL	31.0	2.1	1.0	2.1	0.5
rB6-4-G8-IGFR	SAVLEYLLAYFARTGAA	38.7	5.5	2.6	2.1	0.5
rB6-3-G5-IGFR	DRALGPLWRYFMVNNQG	32.8	2.0	1.0	2.0	0.5
rB6-4-C9-IGFR	WRILDRLLAYFKESQGD	37.6	30.2	15.1	2.0	0.5
rB6-4-D11-IGFR	DDVLTILFQYFRASTGV	36.6	5.5	3.0	1.8	0.5
rB6-4-B11-IGFR	FDVLTWLGRYF*MNTGK	25.2	4.2	2.4	1.8	0.6
rB6-4-E11-IGFR	RDVLDGLREYFRASVGG	38.1	29.8	22.2	1.3	0.7
rB6-3-G3-IGFR	IKTINDLLAYFRGDLDV	31.6	8.7	7.2	1.2	0.8
rB6-4-H12-IGFR	DEALLWLMRYFRGSFSP	40.1	2.8	2.5	1.1	0.9
rB6-4-G12-IGFR	ESPLDALRAYFSGRRNW	36.7	32.5	31.4	1.0	1.0
rB6-3-C4-IGFR	IQSL*DLLOQYFVSSPSV	37.1	6.2	13.5	0.5	2.2
	GGILD?ILODYFRSTDVG					

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FIG. 3D-2

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
R20β-4-F8-IR	XXXXXXXXXXXXXXXXXXXXX	39.1	1.8	27.7	0.1	15.4
	HLCVLEELFWGASLFGYCSG					

FIG. 4A

Cl ne	Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
rB6-4-B10-IGFR	RPVLGWLFDFVASDPM	XXXXXXXXXXXXXX	33.1	26.9	1.0	26.9	<0.1
rB6-3-E6-IGFR	RWPLSALMDYFRRSDGV	XXXXXXXXXXXXXX	37.6	26.6	1.0	26.6	<0.1
rB6-4-B9-IGFR	DGVLASLWRYFVSGGTL	XXXXXXXXXXXXXX	39.2	26.3	1.0	26.3	<0.1
rB6-3-F5-IGFR	DRQLGWLWDYFHLTDLP	XXXXXXXXXXXXXX	33.2	15.6	1.0	15.6	0.1
rB6-3-B6-IGFR	DGILGLIMAYFVES?RV	XXXXXXXXXXXXXX	37.4	13.3	1.0	13.3	0.1
rB6-3-D4-IGFR	QDLLGRIMLYFAETDTV	XXXXXXXXXXXXXX	31.2	20.7	2.0	10.4	0.1
rB6-4-D10-IGFR	SGVLADLFRYFQRHPWP	XXXXXXXXXXXXXX	31.7	10.1	1.0	10.1	0.1
rB6-3-D6-IGFR	DPPLGGLWTFYFSRSDPG	XXXXXXXXXXXXXX	33.9	9.9	1.0	9.9	0.1
rB6-4-F9-IGFR	DSVLSLSYFASGDIA	XXXXXXXXXXXXXX	34.3	28.3	3.0	9.4	0.1
rB6-3-E1-IGFR	DGVLAALEAYFRHGPRD	XXXXXXXXXXXXXX	30.5	9.3	1.0	9.3	0.1
rB6-3-B2-IGFR	DEILGALYSYFSLSGGA	XXXXXXXXXXXXXX	22.2	8.8	1.0	8.8	0.1
rB6-3-D7-IGFR	QDVLGALQRYFASGEPP	XXXXXXXXXXXXXX	31.2	7.6	1.0	7.6	0.1
rB6-4-C11-IGFR	DSVLQYLLNHFGADSKQ	XXXXXXXXXXXXXX	33.7	7.6	1.0	7.6	0.1
rB6-4-F12-IGFR	NEVLEGLFSYFVY?ANG	XXXXXXXXXXXXXX	38.1	7.3	1.0	7.3	0.1
rB6-4-F7-IGFR	SGILGQLLRYFKGAGGG	XXXXXXXXXXXXXX	38.6	7.3	1.0	7.3	0.1
rB6-3-G6-IGFR	DELLDRLWQYFQVGGDL	XXXXXXXXXXXXXX	34.0	7.1	1.0	7.1	0.1
rB6-4-E8-IGFR	PGILLDLWRYFASAPDQ	XXXXXXXXXXXXXX	37.6	6.9	1.0	6.9	0.1
rB6-4-G10-IGFR	DSVLLDLYEYFSSGSSG	XXXXXXXXXXXXXX	34.9	14.5	2.2	6.6	0.2
rB6-4-B12-IGFR	DGMLSRLWEYFAGTNVP	XXXXXXXXXXXXXX	36.3	28.9	4.5	6.4	0.2
rB6-3-B5-IGFR	DVILGGLWDYFASGGGH	XXXXXXXXXXXXXX	17.2	6.1	1.0	6.1	0.2
rB6-3-C5-IGFR	GGVLAALERYFRVSAGD	XXXXXXXXXXXXXX	38.7	15.8	2.9	5.4	0.2
rB6-4-B8-IGFR	DEVLGRLWAYFAQESLG	XXXXXXXXXXXXXX	31.9	22.0	4.1	5.4	0.2
rB6-3-H2-IGFR	DGILQSLWDYFARSPVG	XXXXXXXXXXXXXX	31.8	22.4	4.2	5.3	0.2
rB6-3-E5-IGFR	VDILSELWDYFRRGEEG	XXXXXXXXXXXXXX	37.0	20.5	4.0	5.1	0.2
rB6-3-B3-IGFR	DKVLRLLGEYFATHSKG	XXXXXXXXXXXXXX	31.7	4.8	1.0	4.8	0.2
rB6-4-G7-IGFR	QGFLAWLRDIFYASGTRS	XXXXXXXXXXXXXX	37.4	10.0	2.1	4.8	0.2
rB6-3-A3-IGFR	QDVLSRLLSYFMNGNDV	XXXXXXXXXXXXXX	27.2	4.7	1.0	4.7	0.2
rB6-4-E9-IGFR	DGVLSKIWEYFKIOGND	XXXXXXXXXXXXXX	37.3	20.1	4.8	4.2	0.2

FIG. 3D-1

Cl ne	Design	Sequence IR/IGFR	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
rB6-4-E7-IR		LDPI ¹ DALLQYFWSVPGH	26.4	1.0	15.5	0.1	15.5
rB6-4-A12-IR		LDALDRLMRYFEERPSL	34.9	1.0	12.0	0.1	12.0
rB6-3-E6-IR		ADELEWLLDYFMHQPRP	9.0	1.0	4.8	0.2	4.8
rB6-4-E11-IR		DQELGWLRGYFEWTARD	31.2	1.6	5.9	0.3	3.7
rB6-4-F12-IR		DGVLEELFSYFSATVGP	30.4	1.0	3.4	0.3	3.4
rB6-4-D11-IR		PMNLSELWDYFRLLKPGR	41.9	15.7	30.2	0.5	1.9
rB6-4-A8-IR		DSILRELRDYFAPYSHC	25.6	2.4	4.6	0.5	1.9
rB6-4-E8-IR		DDALEWLLNYFQNGHVQ	33.0	9.7	15.9	0.6	1.6
rB6-4-B9-IR		GDILDALLRYFEFGVDT	42.7	17.2	21.7	0.8	1.3
rB6-3-A6-IR		GDQIAWLLAYFQSDGSD	32.3	2.9	2.8	1.0	1.0
rB6-4-C7-IR		DGVLEGLLSYFTSTNSH	31.4	2.6	2.3	1.1	0.9
rB6-4-H12-IR		ARPLDWLLDYFKQGARG	26.0	10.0	7.2	1.4	0.7
rB6-3-C6-IR		DDMLRQLWL ² YFEASAGG	34.2	19.1	12.8	1.5	0.7
rB6-4-G12-IR		DPWLAWLGRYFGETATG	37.7	6.1	3.1	2.0	0.5
rB6-4-G12-IR		DPTLFGLLRYFQESGIA	33.3	7.6	3.5	2.2	0.5
rB6-4-C11-IR		MDPLRGLLMYFSQGGLV	26.6	18.7	4.7	4.0	0.3
rB6-4-G8-IR		DGLLWQLWDYFALSEHR	37.3	7.4	1.3	5.7	0.2
rB6-4-B8-IR		DNWLSALMAYFMGSGES	31.1	28.6	1.0	28.6	<0.1
rB6-4-D7-IR		DDVLN ³ YLLGYFRQSDGL	24.1	29.4	1.0	29.4	<0.1

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FIG. 3C

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
R20-4-F9-IGFR	PLAELWAYFEHSEQGRSSAH	33.1	19.3	1.0	19.3	0.1
R20-4-H4-IGFR	PVLSGLLRYPAGEPLGQPOS	24.1	5.6	3.2	1.8	0.6
R20-4-F9-IGFR	GGYLDLWLHYFRDQALQPW	2.5	2.4	1.4	1.7	0.6
R20-4-D6-IGFR	VDQRQGGWLLALENYFRSTV	6.1	2.9	1.9	1.5	0.7
R20-4-G2-IGFR	DVPAGGLLRQMWVYFRDSDP	6.3	2.2	2.0	1.1	0.9

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FIG. 3A

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
20C-3-F3-IGFR	RRVACTQADGLLCESDPLKALLSYF	35.5	32.8	17.9	1.8	0.5

FIG. 3B

Clone Design	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
NNRPβ-4-H11-IR	XXXXXXXXFYRYFXLLXXXXXXXX	2.3	12.4	9.0	1.4	0.7
NNRPα-1-B1-IR	DYTA FYGYFNLLRTSG	18.8	18.7	11.5	1.6	0.6
NNRPα-2-D7-IR	FQSSFYGYFESLLMSYK	21.0	21.8	13.2	1.6	0.6
NNRPα-2-D8-IR	DTNA FYRYFEGLLWSEH	20.2	19.8	12.2	1.6	0.6
NNRPα-3-E1-IR	GGSSFYRYFEQLLAQWE	20.0	20.5	12.9	1.6	0.6
NNRPα-3-E5-IR	SQGGFYRYFEKLLDEV	20.0	24.5	15.5	1.6	0.6
NNRPα-3-H3-IR	RSGLFYRYFEELLQAI	19.8	19.1	12.2	1.6	0.6
NNRPα-3-H5-IR	QGGGFYHYFLSLLEEVG	19.9	18.0	11.1	1.6	0.6
NNRPα-1-A3-IR	WRGA FYRYFQTLLSDEG	24.4	14.9	7.9	1.9	0.5
NNRPα-3-E6-IR	AAGFYGYFYSLLGDQT	23.1	19.0	9.6	2.0	0.5
NNRPα-3-F9-IR	RNSGFYRYFQHLVSEWE	21.1	18.8	9.6	1.9	0.56
NNRPα-3-G6-IR	QHRLFYSYFAELLGRDT	22.4	17.7	9.0	2.0	0.5
NNRPα-3-H9-IR	QIDEFYRYFADQLRGFA	18.3	13.1	6.9	1.9	0.500
NNRPα-1-A8-IR	LGGFYRYFNLLVMGSG	16.9	13.8	5.7	2.4	0.4
NNRPα-1-A9-IR	GDRA FYRYFQRLQEGWG	16.5	15.2	5.6	2.7	0.4
NNRPα-2-D6-IR	CEDAFYRYFVNLLGGGC	19.4	18.5	6.8	2.7	0.4
NNRPα-3-F6-IR	NYSQFYRYFEMLLLEGDV	22.8	19.5	7.9	2.5	0.4
NNRPα-3-H2-IR	VGDA FYRYFQGLLRQDQ	19.9	18.9	8.5	2.2	0.4
NNRPα-1-B6-IR	MHGS FYRYFQDLLQAPP	14.1	11.5	3.9	3.0	0.3
NNRPα-2-C6-IR	DVGDFYRYFGLLLTSDR	18.4	19.2	4.1	4.7	0.2
NNRPγ-4-A1-IR	NSAAFYGYFSQLLAQIR	9.7	10.9	1.8	6.0	0.2
NNRPγ-4-A7-IR	IIGGFYSYFNSVLRIGT	2.5	6.3	1.3	4.9	0.2
NNRPγ-4-A9-IR	REDPFYSYFVNLLGASA	3.0	10.0	1.4	7.1	0.1
NNRPγ-4-B11-IR	EGSGFYGYFFSLLGLQG	4.1	18.7	1.2	15.5	0.1
	LKDGFDYDFWQRLHLS					

FIG. 2P-5

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Cl ne Design	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
NNRPγ-4-A12-IR	XXXXXXXXFYRYFXKLLXXXXXXXXX	4.9	16.3	14.9	1.1	0.9
NNRPγ-4-B8-IR	AFYRYFRDLLFSGF	6.1	21.3	19.9	1.1	0.9
NNRPα-1-A5-IR	DDRGFYRYFESLLLGSS	2.3	1.4	1.1	1.2	0.8
NNRPα-1-B7-IR	ISTSFYQYIAGLLRGDR	19.2	15.7	12.4	1.3	0.8
NNRPα-2-C7-IR	GSSGFYRYFNMMLSQT	19.6	20.0	16.5	1.2	0.8
NNRPα-2-C11-IR	GDRGFYRYFEGLLASVG	20.1	20.0	16.3	1.2	0.8
NNRPα-2-C12-IR	NSAAFYRYFEQLLREVE	14.3	10.1	8.5	1.2	0.8
NNRPα-2-D12-IR	LSDFYRYFEQLMGARS	11.5	11.4	9.3	1.2	0.8
NNRPα-3-G2-IR	RSTLFYRYFQNLLEEVG	20.8	20.7	16.1	1.3	0.8
NNRPα-3-G8-IR	TRGGFYRYFEDLLQVYS	14.7	11.0	9.2	1.2	0.8
NNRPα-3-G10-IR	GVSGFYRYFQSLLDSSYG	18.8	16.5	13.9	1.2	0.8
NNRPα-3-G11-IR	QNDAFYSYFNSLLQAYT	12.0	10.3	8.5	1.2	0.8
NNRPα-3-G12-IR	RQQDFYRYFRQLLEEV	11.7	11.8	9.3	1.3	0.8
NNRPγ-4-B2-IR	EGSGFYRYFEKLLQSP	22.8	30.3	25.3	1.2	0.8
NNRPα-1-B8-IR	RHKAFYRYFEELLQKNV	16.1	10.1	6.9	1.5	0.7
NNRPα-2-C5-IR	GMTRLIVRSTVISRELLHYSL	18.1	19.2	13.7	1.4	0.7
NNRPα-2-C9-IR	QALSFYRYFERLLDEVS	22.9	21.7	16.1	1.3	0.7
NNRPα-2-D2-IR	SKSAFYRYFDELLGNSG	26.1	26.2	17.6	1.5	0.7
NNRPα-2-D5-IR	LGGAFYRYFAQLLNSHV	21.7	21.1	15.4	1.4	0.7
NNRPα-2-D11-IR	INSGFYGYFVQLLSGHQ	12.3	10.8	7.8	1.4	0.7
NNRPα-3-E2-IR	SQSSFYRYFESLLEDNP	24.4	25.5	18.3	1.4	0.7
NNRPα-3-E4-IR	ADGGFYGYFAALLGSVS	23.0	22.3	14.7	1.5	0.7
NNRPα-3-F4-IR	QNGSFYRYFIALLGDSG	24.9	25.1	17.6	1.4	0.7
NNRPα-3-G4-IR	WDTGFYRYFIELLEDSD	20.9	20.4	14.1	1.5	0.7
NNRPα-3-H4-IR	HPRDFYRYFERLLNQVD	17.7	17.6	11.6	1.5	0.7
NNRPα-4-E12-IR	DGGAFYRYFMDLLGAHE	4.3	15.4	10.8	1.4	0.7
NNRPβ-4-G11-IR	AGRGFYRYFEHLLAGRE	6.6	18.4	13.1	1.4	0.7
NNRPβ-4-H6-IR	SSRGFYRYFRELLADSW	2.2	16.1	11.7	1.4	0.7
	KYSGFYRYFENALLGRRE					

FIG. 2P-4

Clone Design	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
NNRPγ-4-A10-IR	SGFAFYQYFQELLAGHD	7.6	20.3	22.0	0.9	1.1
NNRPγ-4-B6-IR	GDGGFYGYFASLLSGEG	12.2	22.3	24.2	0.9	1.1
NNRPγ-4-B9-IR	EANGFYRYFYDLLQDFG	6.7	22.9	25.9	0.9	1.1
NNRPα-4-C8-IR	AVNGFYRYFNRLLESVE	8.5	16.3	16.0	1.0	1.0
NNRPα-4-C9-IR	QQDGFYRYFLOLLDEVA	5.6	20.7	19.9	1.0	1.0
NNRPα-4-C10-IR	ISQGFYGYFSRLIQDTE	6.7	16.5	17.2	1.0	1.0
NNRPα-4-E11-IR	YSTGFYRYFLLDGLMP	6.0	20.3	20.9	1.0	1.0
NNRPβ-4-F11-IR	PNGDFYRYFLLDGLSVG	7.7	21.8	21.9	1.0	1.0
NNRPβ-4-G2-IR	RHQAFYSYFRDLPRECP	19.1	24.7	25.6	1.0	1.0
NNRPβ-4-G9-IR	ETEGFYRYFEELLAQVA	7.8	27.3	26.4	1.0	1.0
NNRPβ-4-H7-IR	AGDRFYDYFDRLLADYD	2.6	26.6	27.9	1.0	1.0
NNRPβ-4-H8-IR	GGSGFYRYFWGLLAEOE	3.6	23.0	24.1	1.0	1.0
NNRPγ-4-B1-IR	LLNRLYRYFAGAEWFG	17.6	24.5	23.4	1.0	1.0
NNRPγ-4-B10-IR	DGSGFYRYFEMLLGSGL	5.5	18.3	19.0	1.0	1.0
NNRPα-1-B3-IR	RDMAFYRYFSHLLESFQ	16.4	13.4	12.7	1.1	0.9
NNRPα-2-C2-IR	GNAGFYRISRILWQTE	22.5	24.4	21.3	1.1	0.9
NNRPα-2-C3-IR	GNAGFYRYFADIMAGYE	19.6	21.7	19.7	1.1	0.9
NNRPα-2-D10-IR	YQAAFYRYFATLLSTTD	17.8	6.3	5.4	1.2	0.9
NNRPα-3-E11-IR	GGLGFYRYFQLLLGSSG	12.9	10.8	9.6	1.1	0.9
NNRPα-3-F5-IR	DGSGFYGYFDFVLRQFE	25.1	18.3	17.0	1.1	0.9
NNRPα-3-F8-IR	VGSGFYRYFDQLLGMYG	22.2	15.7	13.9	1.1	0.9
NNRPα-3-F10-IR	YGTDFYLYFDQLLLOYG	20.5	14.6	13.1	1.1	0.9
NNRPα-3-G7-IR	FNSSFYLYFRDLLNTVG	21.0	18.3	15.6	1.2	0.9
NNRPα-4-C2-IR	RAAGFYRYFEDLLGARG	25.5	25.1	23.3	1.1	0.9
NNRPα-4-D12-IR	TCAGFYRYFIDLLGETG	14.7	19.7	18.5	1.1	0.9
NNRPβ-4-G3-IR	RDLEFYGYFQELLRLNF	14.6	27.8	25.7	1.1	0.9
NNRPβ-4-G4-IR	GMGPFYRYFIDLLRESD	20.0	28.6	24.9	1.1	0.9
NNRPγ-4-A5-IR	HGDGFYQYFMEVLRLLQN	17.0	29.0	27.3	1.1	0.9

FIG. 2P-3

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFR	IGFR/IR	IR/IGFR
NNRPβ-4-G10-IR	XXXXXXXXFYRYFYFALLXXXXXXXX	8.8	8.7	11.7	0.7
NNRPβ-4-H2-IR	SSREFYSYFSGLLTAL	4.9	19.9	25.3	0.8
NNRPα-4-C1-IR	TGRGFYRYFEGLEDDWM	15.5	18.0	21.1	0.9
NNRPα-4-C5-IR	SGSWFYRYFEELLQSG	18.0	23.3	26.9	0.9
NNRPα-4-C6-IR	GRGGFYQYFLDLLQTEA	7.8	13.6	15.7	0.9
NNRPα-4-C12-IR	GQNGFYRYFDLILADWV	12.3	16.7	19.9	0.8
NNRPα-4-D7-IR	FAGSFYRYFEQILLSEQ	10.2	23.7	27.9	0.8
NNRPα-4-D11-IR	DPNAFYRYFEGLLWREH	5.4	19.3	22.3	0.9
NNRPβ-4-F1-IR	?GLNFYRYFVGLLTDTL	21.7	23.0	28.6	0.8
NNRPβ-4-F9-IR	RHINFYGYFDDLLATWH	10.1	18.4	22.5	0.8
NNRPβ-4-F12-IR	FHRGFYRYFINLLSGDA	4.5	13.5	16.6	0.8
NNRPγ-4-A3-IR	MGSSFYRYFETLLGQGL	16.4	22.3	26.8	0.8
NNRPα-1-A7-IR	GS�DFYSYFWRGLGIP	16.9	1.2	1.3	0.9
NNRPα-4-C11-IR	STVSFYRYFYALLQSPC	7.8	19.7	21.2	0.9
NNRPα-4-D8-IR	LGGYFYRYFEDLLNHQS	6.9	17.6	20.1	0.9
NNRPα-4-D10-IR	DHRGFYRYFELYQLAGNV	6.4	17.2	19.5	0.9
NNRPα-4-E5-IR	EYSGFYGYFNHLLGSLG	13.2	26.1	27.6	0.9
NNRPα-4-E8-IR	TSNWFYQYFTDLLAGED	8.7	22.9	24.2	0.9
NNRPα-4-E10-IR	SSGGFYRYFSQLLTEMN	3.5	12.4	13.2	0.9
NNRPβ-4-F8-IR	VHGEFYRYFESLLRETF	8.1	22.9	25.2	0.9
NNRPβ-4-F10-IR	SDEGFYRYFAQLLYGVT	5.3	17.9	19.1	0.9
NNRPβ-4-G8-IR	ETGGFYGYFQALLATYH	10.6	27.2	28.9	0.9
NNRPβ-4-H3-IR	GDRGFYRYFEWLLNDEG	3.9	24.2	25.7	0.9
NNRPβ-4-H9-IR	DGGA FYRYFEALLGEMG	4.1	26.5	29.3	0.9
NNRPβ-4-H10-IR	DGGA FYRYFEALLGELD	3.4	22.3	24.6	0.9
NNRPγ-4-A6-IR	WHSDFYRYFLLSLQEDG	14.9	25.8	27.6	0.9
NNRPγ-4-A8-IR	EEEGFYGYFYRLLGVER	9.8	22.8	24.7	0.9
	MDAGFYGYFSDLLANWG				

FIG. 2P-2

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGFR --	IR --	IGFR/IR --
NNRPβ-4-G6-IR	RWPNFYGYFESLLTHFS	10.1	1.9	20.1	0.1
NNRPβ-4-F3-IR	HYNAFYEFQVLLAETW	8.6	1.3	13.6	0.1
NNRPα-2-C1-IR	EGWDFYSYFSGLLASVT	19.7	2.0	10.9	0.2
NNRPα-4-E1-IR	LDRQFYRYFQDLLVGFM	11.5	6.5	21.2	0.3
NNRPα-3-H6-IR	WGRSFYRYFETLLAQGI	19.1	2.1	6.0	0.3
NNRPβ-4-F7-IR	RREGFYHYFQSLIDEYG	0.7	0.9	2.3	0.4
NNRPα-2-D1-IR	GGGQFYRYFIDMLVLDI	18.4	1.5	3.7	0.4
NNRPα-1-A1-IR	PTGPFDRYFARRLVWRG	15.2	1.3	3.1	0.4
NNRPα-2-C10-IR	RGGAFYRYFEGLLSQHN	18.8	3.8	8.8	0.4
NNRPα-3-G1-IR	WRDPFYRYFQDLLEGER	18.9	4.2	8.6	0.5
NNRPα-4-C3-IR	WGGEFYRYFVQLLSSSD	17.9	12.9	25.7	0.5
NNRPα-4-D1-IR	GRESFYGYFLDLLQETV	16.2	12.7	23.2	0.5
NNRPβ-4-F4-IR	GHAIFYGYFQGLLDSYL	19.5	16.0	25.6	0.6
NNRPα-1-B2-IR	GGEAFYRYFWGLLTEWE	14.8	8.4	12.9	0.7
NNRPα-1-B4-IR	LSSGFYRYFTGLLSDGQ	19.1	6.3	9.2	0.7
NNRPα-4-D9-IR	DPGAFYRYFAQLMDTWN	7.6	16.9	25.7	0.7
NNRPβ-4-F2-IR	KHEQFYRYFRNLLGAMS	21.6	20.9	30.8	0.7
NNRPβ-4-H12-IR	RDGAFYRYFEDLLIAVD	5.2	13.8	20.0	0.7
NNRPα-4-E7-IR	RGNRFYRYFEYLLRDYG	9.4	21.9	29.7	0.7
NNRPα-1-B5-IR	ELGDFYRYFQLLADNH	14.1	5.4	7.1	0.8
NNRPα-4-C4-IR	AQDAFYSYFSVLLGEHL	17.6	17.6	22.3	0.8
NNRPα-4-C7-IR	IGVNFYRYFEKLLLDEF	4.5	11.2	14.9	0.8
NNRPα-4-D3-IR	TDSQFYSYFESLLETFG	16.4	13.5	17.9	0.8

FIG. 2P-1

Clon	Design	Sequence	Ratios over Background		Comparisons		
			E-Tag	IGFR	IR	IGFR/IR	IR/IGFR
20E2B-3-D3-IGFR	20E2B-3-D3-IGFR	XXXXXXXXXXhXXXXXXXX	--	--	--	--	--
20E2B-4-H8-IGFR	20E2B-4-H8-IGFR	CGRRDFYGGIICLLGQKGVV	21.0	16.1	2.6	6.2	0.2
20E2B-3-E9-IGFR	20E2B-3-E9-IGFR	PAGPCGYCGLGLLLHGDQSP	7.2	5.3	0.9	5.9	0.2
20E2B-4-H9-IGFR	20E2B-4-H9-IGFR	QAAPQDFYQGLWLLIHRDPTM	14.7	16.2	2.8	5.8	0.2
20E2B-1-A8-IGFR	20E2B-1-A8-IGFR	RCQGTGFYTCIQELIGFGDPD	4.5	5.2	0.9	5.6	0.2
20E2B-4-H11-IGFR	20E2B-4-H11-IGFR	TLSRPTFYDWLEMLVTHGQGG	16.1	4.4	0.9	5.0	0.2
20E2B-3-C9-IGFR	20E2B-3-C9-IGFR	STHSRAFYDAIAQLVGSVLGP	10.7	11.0	2.3	4.8	0.2
20E2B-3-E6-IGFR	20E2B-3-E6-IGFR	RQGGSFYELLCGLVGGEVCV	17.9	19.7	4.2	4.6	0.2
20E2B-3-E11-IGFR	20E2B-3-E11-IGFR	RQASGFYRALHDLMLRTQDY	24.5	21.6	4.7	4.6	0.2
20E2B-4-G8-IGFR	20E2B-4-G8-IGFR	SRANLFFYMGLSQLLRDNRGL	16.5	7.7	1.9	4.1	0.2
20E2B-4-H10-IGFR	20E2B-4-H10-IGFR	GRALDPFYDQLRDLVARSGGG	11.1	14.9	3.7	4.1	0.2
20E2B-3-E7-IGFR	20E2B-3-E7-IGFR	EASCRTFYCGLMALIGGDDQR	2.2	2.5	0.8	3.1	0.3
20E2B-3-C12-IGFR	20E2B-3-C12-IGFR	QNGCKDFYCLIDNLIRYGPGG	14.4	8.8	3.0	3.0	0.3
20E2B-4-G12-IGFR	20E2B-4-G12-IGFR	QHSRRTFYDCIRVLMDDGQLG	6.2	6.4	2.2	2.9	0.3
20E2B-4-G3-IGFR	20E2B-4-G3-IGFR	LDSRRGFYDWIKALIGDRDVQ	9.6	10.0	3.8	2.6	0.4
20E2B-3-E4-IGFR	20E2B-3-E4-IGFR	CQKGDYAGLVCLLRERASQ	27.2	23.8	9.1	2.6	0.4
20E2B-3-E2-IGFR	20E2B-3-E2-IGFR	GGSQSFYDVMCMILLQLDPTC	24.9	22.3	8.9	2.5	0.4
20E2B-2-B4-IGFR	20E2B-2-B4-IGFR	VESDVSYEGLMRLVWVGQGG	18.6	20.2	8.7	2.3	0.4
20E2B-3-C5-IGFR	20E2B-3-C5-IGFR	ERAGDLFYQWFERLVAGHGLE	5.8	2.3	1.0	2.2	0.5
20E2B-3-C5-IGFR	20E2B-3-C5-IGFR	RMPSGSFYQGIYELVTRQGGF	6.3	2.0	0.9	2.2	0.5

FIG. 20-2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IR	IGFR/IR	IR/IGFR
20E2B-1-A6-IGFR	xxxxxxfyyxxhxxhxxxxxx	18.6	--	--	--
20E2B-3-C6-IGFR	GVRAMSFYDALVSVLGLGPSG	18.1	1.1	16.8	0.1
20E2B-4-H3-IGFR	VEGRGLFYDLLRQLARRQNG	17.9	1.1	14.8	0.1
20E2B-3-C2-IGFR	KLHNLMEYYQLQRLVWGAGLG	11.2	14.8	13.9	0.1
20E2B-3-E3-IGFR	GNGDGMFYQLLSLLVGRDMHV	13.1	8.9	13.8	0.1
20E2B-4-H12-IGFR	PDLHKGFYAQLAQLLRQLLS	22.4	16.3	13.1	0.1
20E2B-3-D2-IGFR	YSCGDGFYSLLSDLLGGQFRC	6.5	9.7	12.8	0.1
20E2B-3-D8-IGFR	IQQLTFYDLLHRLVRSELGS	20.7	12.4	11.7	0.1
20E2B-3-E8-IGFR	GGTEVDFYRALERLVRGQLGL	20.4	17.7	11.3	0.1
20E2B-4-F8-IGFR	LRIANLFYQRLWDLAFGGG	15.7	16.7	11.1	0.1
20E2B-1-A11-IGFR	PVGVOGFYEGLSRLVLGRGW	12.3	7.3	9.7	0.1
20E2B-3-D4-IGFR	RFSTDGFYQYLLALVGGPVG	15.0	9.5	9.7	0.1
20E2B-2-B11-IGFR	NSRDGGFYQLERLLGFPVTG	8.1	7.9	9.6	0.1
20E2B-3-C8-IGFR	VVTPVNFYRALEALVRQRLG	13.9	10.6	9.4	0.1
20E2B-2-B2-IGFR	QPAPDGFYSALMKLIGRGVS	18.5	15.6	8.9	0.1
20E2B-4-F10-IGFR	PGTDLGFYQALRCVVIQACD	11.7	4.9	8.1	0.1
20E2B-4-F9-IGFR	AQPCGGFYGLLEQLVGRSVCD	19.0	17.3	7.8	0.1
20E2B-3-D11-IGFR	QPDHSYFYSLLQELVGSERL	11.9	14.7	7.7	0.1
20E2B-3-C11-IGFR	LGVTDFYALGYLIHGVGF	14.3	12.2	7.6	0.1
20E2B-2-B3-IGFR	CMMQDGFYAGLGCLLTAGEGR	15.3	15.4	7.5	0.1
20E2B-3-D12-IGFR	ICTGQGFYQVLCGLLRGTSAR	9.1	5.3	7.4	0.1
20E2B-3-E12-IGFR	QGNVLDYFGWIGRLLAKQGS	10.3	6.2	7.3	0.1
20E2B-2-B8-IGFR	VATSQGFYSGLSELLQGGNV	13.9	6.0	7.3	0.1
20E2B-4-G11-IGFR	IWATGDFYRLLSQLVMGRVGT	17.4	5.7	7.2	0.1
20E2B-3-D6-IGFR	RQGTGSFYLMLEQLLVGARGP	8.9	4.5	7.0	0.1
20E2B-2-B7-IGFR	DSVGNFYQLLESIVGGHVG	20.7	17.8	6.9	0.1
20E2B-3-C4-IGFR	LSSDGFYRALNLLQLQSGR	18.0	6.1	6.7	0.1
	ASSASGFYELLQRLAGLGLV	23.4	20.4	6.2	0.2

FIG. 20-1

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	--
20E2B α -3-B6-IR	SGPMVGFYRGLFSLSPEDLQ	39.7	34.9	31.5	1.1	0.9	
20E2B α -3-D1-IR	LAEPDSFYNNWIAQLLEGEFAG	41.6	35.1	31.7	1.1	0.9	
20E2B α -3-A9-IR	FSGCDNFYSCIQSLWLGPGGV	37.3	35.1	32.4	1.1	0.9	
20E2B α -3-C4-IR	QVFCDNFYHCIEITLLGVGQTP	39.6	36.3	33.4	1.1	0.9	
20E2B α -4-F3-IR	RGRDNQFYHGLWALLIGSGLE	37.5	36.6	33.6	1.1	0.9	
20E2B α -4-F4-IR	VSGRGGFYDAIRDLLIGPRDQG	37.2	36.9	33.7	1.1	0.9	
20E2B α -3-D4-IR	PVILDDFYVALCOLMVQDCFF	42.1	38.0	34.5	1.1	0.9	
20E2B α -4-E4-IR	PDIADPFYAFFQGLLRADTPI	40.6	38.4	35.5	1.1	0.9	
20E2B α -4-G10-IR	VAQCTDFYACIRSLVRSGPSG	32.9	31.3	27.1	1.2	0.9	58/200
20E2B α -3-D11-IR	CSQLVSFYLGMDCLLGRGGTQ	34.0	32.5	27.9	1.2	0.9	
20E2B α -3-C8-IR	PLACADFYQCLSDLIRGGPAW	39.2	33.0	28.2	1.2	0.9	
20E2B α -4-F2-IR	VVICTGFYDCIYQIVGSHEEM	38.7	37.6	32.3	1.2	0.9	
20E2B α -4-H12-IR	CVDRRTFYEGLOCLLGGATGD	32.3	30.4	25.8	1.2	0.8	
20E2B α -4-E1-IR	VNLRDPFYQWIEALMDSAGGE	39.2	40.2	32.3	1.2	0.8	
20E2B α -4-H8-IR	LTSSTSFYDALFCLAGLQLCG	37.6	34.8	27.0	1.3	0.8	
20E2B α -3-B4-IR	DFDSSPFYRGLRQLLESRSFP	39.9	34.9	25.9	1.3	0.7	
20E2B α -4-E2-IR	HEAGWTFYDAIQCLVGGWCSK	38.8	36.3	23.5	1.5	0.6	
20E2B α -4-H1-IR	CQWRSFYHAVSCLLGPDDPD	40.8	33.6	20.2	1.7	0.6	
20E2B α -3-A10-IR	MVDRDPFYQGLRDLIGRQEKG	32.8	32.6	18.5	1.8	0.6	
20E2B α -3-D3-IR	LGRGGFYRGLQDLIGTQWPR	41.9	29.5	5.6	5.3	0.2	

FIG. 2N-2

Clone Design Parental	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
20E2Bα-3-B3-IR	XXXXXXXXXXXXXXXXXXXX FYDAIDQLVRGSAAGGTRD	30.6	15.1	4.2	3.6 0.3
20E2Bα-4-F12-IR	AGVNAGFYRYFSTLLDWDQG	33.5	1.2	23.5	0.1 20.0
20E2Bα-3-B8-IR	SVKEVQFYRYFYDLLQSEEG	35.5	5.9	27.8	0.2 4.7
20E2Bα-3-D2-IR	IEVTQPFYDYFQQLRLYGND	39.3	18.2	36.5	0.5 2.0
20E2Bα-3-A5-IR	VQCRADFYSFACIVGRPGSR	42.6	19.7	26.7	0.7 1.4
20E2Bα-3-A3-IR	RNYPIGFYQFFHELVISSGGG	36.9	22.7	24.5	0.9 1.1
20E2Bα-4-E9-IR	DLGNSFYGLRLRLVLQDAVG	39.9	33.5	35.5	0.9 1.1
20E2Bα-4-G8-IR	CKDQPDFYMGIKCLISGGGSV	32.8	29.6	28.6	1.0 1.0
20E2Bα-4-F9-IR	ACEGGSFYGCQSIMSVEGNN	37.5	30.5	30.9	1.0 1.0
20E2Bα-4-E7-IR	AVHEDGFYDMLRKLLSEGDS	35.6	32.5	31.1	1.0 1.0
20E2Bα-3-D5-IR	LARNDEFYRYFEQLVFGDTEG	36.0	31.6	31.2	1.0 1.0
20E2Bα-4-F7-IR	ATCASSFYAQLNCLLSDFDVM	39.5	33.1	31.8	1.0 1.0
20E2Bα-3-B12-IR	VQACQNFYDCLNTLLLLDLGG	36.6	32.9	32.5	1.0 1.0
20E2Bα-3-B7-IR	IRGADQFYQFFRELLEGSVGE	37.0	33.4	33.5	1.0 1.0
20E2Bα-3-B5-IR	RAGSRGFYEFFENLLRVGAGG	36.9	34.9	34.2	1.0 1.0
20E2Bα-4-G1-IR	AORCADFYACIEELLAPGSWR	40.4	37.1	36.3	1.0 1.0
20E2Bα-4-G11-IR	PGGEGFYQGLQRLILGADGG	41.6	36.4	34.5	1.1 1.0
20E2Bα-4-E10-IR	QKRSEAFYDWIADLLGQETSG	38.5	28.9	26.5	1.1 0.9
20E2Bα-4-F11-IR	WGLRDDFYRGIRCLVQWSEGC	33.2	30.1	27.8	1.1 0.9
20E2Bα-4-H11-IR	DSTVCGFYCRLAQLVAEGGSP	35.4	30.5	28.0	1.1 0.9
20E2Bα-4-H3-IR	QHSCTRFFYDCTIRVLMDDGQLG	32.5	29.5	28.0	1.1 0.9
20E2Bα-4-H7-IR	WSGNVDFYMIHQQLCGDVCGS	34.8	32.0	28.7	1.1 0.9
	QTVHRDFYAAQLQDLLINDLGF	38.7	34.9	30.5	1.1 0.9
	SSGCQDFYSCMIQLVVTGGGGD	35.3	32.5	30.5	1.1 0.9

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FIG. 2N-1

Ratios over Background		Comparisons	
E-Tag	IGFsR	IGFR/IR	IR/IGFR
--	--	--	--
23.4	22.4	1.1	0.9
21.0	17.0	1.1	0.9
22.0	19.7	1.0	1.0

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Clone	Design	Sequence
20E2A-3-C1-IGFR		XXXXXXXXFYDAIDQLVXXXXXX
20E2A-3-B3-IGFR		GQSPLSFYDAIDQLVRAFPVG
20E2A-3-D8-IGFR		AGQLGGFYIAICQLVGVEYCT
		SAGPLSFYDAIAQLVGPWRL

FIG. 2M-4

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR
20E2A-4-G2-IGFR	XXXXXXXXFYDAIDQLVXXXXXX	--	--	--	--
20E2A-4-G3-IGFR	IGVLGSFYDAIDQLVRQGNR	22.3	17.5	9.9	1.8
20E2A-3-B5-IGFR	RDVADGFYAAIEQLVRQFGL	21.2	12.3	6.9	1.8
20E2A-4-H4-IGFR	VRQAKSFYDAIDQLVRGALRG	24.0	22.7	13.3	1.7
20E2A-4-F8-IGFR	QVFRGSFYDAIDALVRWGGR	22.2	20.6	12.0	1.7
20E2A-3-A6-IGFR	VGAAFSYDAIDQLVGNWSPGS	17.3	17.9	10.7	1.7
20E2A-4-H12-IGFR	PSPVWSFYDAIQQLVRSQGRG	23.8	23.7	15.0	1.6
20E2A-3-B12-IGFR	PVSATSFYDAINQLVRMGRG	25.1	23.5	14.2	1.6
20E2A-3-B8-IGFR	VMRDRFYDAIEQLVGRIGV	27.6	21.9	13.6	1.6
20E2A-3-C8-IGFR	TTYVNSFYDALQQLLGGDADV	21.5	19.0	12.2	1.6
20E2A-4-H10-IGFR	LSNMITYDAINQLVGHVQSL	23.2	17.7	11.4	1.6
20E2A-3-C9-IGFR	ASSRLSFYDAIEQLIKWSPGP	25.3	23.8	16.2	1.5
20E2A-4-H2-IGFR	WDLVDSFYDAIDQLVGRVPG	25.4	21.8	14.6	1.5
20E2A-3-B6-IGFR	FAFVGSFYDALAQQLVAQGRS	21.8	20.1	13.0	1.5
20E2A-4-G9-IGFR	EDQPN SFYDAIRQLVMGRISP	20.3	18.1	11.8	1.5
20E2A-4-H6-IGFR	SVGPRSFYDAIDQLVGGAWVG	26.0	16.1	10.8	1.5
20E2A-4-H9-IGFR	KFRVYTFYDAIDQLVNQGRGR	21.9	19.6	13.9	1.4
20E2A-4-G10-IGFR	GRGWGSFYDAIDQLVRGLGET	24.9	16.8	11.8	1.4
20E2A-3-A4-IGFR	FTSFHTFYDAIEQLVQGQDGP	25.3	16.5	12.1	1.4
20E2A-3-A7-IGFR	AGSVTSFYDAMEQLVATGSA	16.8	2.5	1.8	1.4
20E2A-4-E12-IGFR	PRESFSFYDAIHQLVTRVRS	26.0	24.9	19.3	1.3
20E2A-3-D1-IGFR	LGRADGFYDAIKQLVGDWGG	23.3	23.1	17.8	1.3
20E2A-3-C6-IGFR	RSGETTFYDALELLVQSGSR	24.0	22.4	17.6	1.3
20E2A-3-D2-IGFR	PVWLFSFYDAIDQLVRKGLGP	23.7	21.7	17.2	1.3
20E2A-4-E8-IGFR	GRRACQTFYDALEQLVGGGALG	21.4	15.1	11.4	1.3
20E2A-4-G6-IGFR	AGPDM SFYDAIDQLVHCCGPF	18.4	13.6	10.4	1.3
20E2A-4-F5-IGFR	HGEKLSFYDAIAQLVGFDIGH	24.7	21.9	17.7	1.2
20E2A-3-C2-IGFR	GYTPVDFYDAIRQLVTGNPG	21.7	21.7	18.2	1.2
20E2A-4-H8-IGFR	EGGFSSFYDALDQLARGRSD	22.5	19.6	15.8	1.2
20E2A-3-A5-IGFR	VGIVRGFYDAIERLVGDTHGQ	24.4	18.5	15.1	1.2
20E2A-3-C11-IGFR	TPGGFSFYDAIQQLVDVLSDS	22.7	15.6	12.6	1.2
	TNAALTTFYDAIEQLVRWGQRD	25.8	24.3	21.2	1.1

FIG. 2M-3

Clon Design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGFsR --	IGFR/IR --	IR/IGFR --
20E2A-4-F2-IGFR	XXXXXXFYDAIDQLVXXXXXX	19.4	19.0	6.8	2.8
20E2A-3-D4-IGFR	TGVFNDFYDAIDQLVGRVRD	16.1	11.8	4.2	2.8
20E2A-3-B10-IGFR	YGSFETFYDAIDQLVRRGSQP	24.0	14.3	5.3	2.7
20E2A-4-E5-IGFR	ROLLDSFYEAIDQLVRSERP	18.1	20.6	7.9	2.6
20E2A-3-D5-IGFR	WPRGDPFYDAMEKLLSQGGGR	15.1	9.3	3.6	2.6
20E2A-4-G11-IGFR	PGLIQSFYDAIDQLVQRGN	20.7	3.3	1.3	2.6
20E2A-3-C12-IGFR	MNVFVSFYDAIDQLVCSLAP	25.9	17.4	7.2	2.4
20E2A-4-G5-IGFR	LDMIGGFYEAIDQLVGGPCG	23.6	14.6	6.0	2.4
20E2A-3-D9-IGFR	RRPCNSFYDAIDQLVGGRT	19.8	12.3	5.1	2.4
20E2A-4-F10-IGFR	FGRRSTFYDLIDQLVGGRT	21.4	21.6	9.3	2.3
20E2A-4-E2-IGFR	LRAPRSFYEAIDQLVGRSVP	22.6	21.3	9.1	2.3
20E2A-4-E3-IGFR	VQRFSSFYDAIDQLVGVPGS	21.0	21.8	10.1	2.2
20E2A-3-C4-IGFR	PSARMGFYDLIDQLVGVPGS	23.5	17.4	7.8	2.2
20E2A-3-C5-IGFR	SLQPHDFYDAIHLVFGHGRF	22.2	17.1	7.7	2.2
20E2A-4-G12-IGFR	ERHGGSFYDAIAQLVQSDRSR	24.3	16.3	7.4	2.2
20E2A-3-C3-IGFR	YQPPGSFYDWIRELVAGPRRE	11.3	2.7	1.2	2.2
20E2A-3-B7-IGFR	FAHASSFYDAIDQLVAKCQSP	22.3	22.6	10.8	2.1
20E2A-4-E10-IGFR	AQSSSGFYEAIDQLVWGRGPG	22.6	19.9	9.4	2.1
20E2A-4-E6-IGFR	TTSGGSFYDAMYQLVWGDWRR	23.0	16.6	7.9	2.1
20E2A-3-D3-IGFR	ARGTAGFYAEIERLVRGQDHG	20.5	15.7	7.6	2.1
20E2A-4-G4-IGFR	PRHAINFYDAIHLVFGPRQ	22.6	14.5	7.0	2.1
20E2A-4-F1-IGFR	QSAHWSFYDAIERLVNMDTNP	19.6	19.9	9.8	2.0
20E2A-4-H7-IGFR	VGVVSSFYDAIDQLVGDWDRGS	23.0	17.1	8.7	2.0
20E2A-3-B9-IGFR	DTLIASFYDAIDQLVRLGRNQ	26.4	22.1	11.5	1.9
20E2A-4-E9-IGFR	FQGTQGFYDAIERLMRRGERP	25.3	20.7	11.1	1.9
20E2A-3-B1-IGFR	WADWGSFYDAIEQLVQRGGV	23.1	17.9	9.5	1.9
20E2A-4-G1-IGFR	EQLSCGFYDAIHLVHGGGLG	22.6	17.7	9.3	1.9
20E2A-4-E7-IGFR	CGQRCSFYDAIDQLVGLPGA	17.2	8.6	4.6	1.9
20E2A-3-D11-IGFR	MMRVDFGYEAIDRLVNEGQAT	16.1	6.1	3.2	1.9
20E2A-3-A3-IGFR	RQATSFYEAIDQLMGSGGV	5.2	3.0	1.5	1.9
20E2A-4-G7-IGFR	GHYFGSFYDAIDQLVAGMLPG	24.4	21.1	11.5	1.8
	PEGVQGFYDALAHLVGGSLFG				

FIG. 2M-2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
Parental	XXXXXXXXFYDAIDQLVXXXXX	--	--	--	--
20E2A-4-F11-IGFR	FYDAIDQLVRSARAGGTRD	30.6	15.1	4.2	3.6 0.3
20E2A-4-F12-IGFR	QGGASAFYDAIDRLRMRIIG	21.3	18.8	1.3	14.6 0.1
20E2A-3-B4-IGFR	AQSGEGFYDALAQVLVSG	23.3	23.9	3.1	7.8 0.1
20E2A-4-F4-IGFR	GHPAVSFYDAIDQLRRRGGG	21.8	16.6	2.4	6.9 0.1
20E2A-3-C7-IGFR	YSDTSFYDAIVQLVRRGASA	20.7	20.0	3.6	5.5 0.2
20E2A-3-C10-IGFR	VGTVAGFYDAIAQLVARASRV	17.6	5.4	1.1	5.1 0.2
20E2A-3-D6-IGFR	RFVWGSFYDAIDQLVQGRWRG	23.3	21.0	4.2	5.0 0.2
20E2A-4-F6-IGFR	RAVGDSFYEAIDQLVRRGGHV	15.1	11.8	2.4	5.0 0.2
20E2A-3-A8-IGFR	LRQLSFYEAIDQLVQWKGA	21.5	19.9	4.3	4.6 0.2
20E2A-4-F9-IGFR	DKFFTSFYDAIDQLVQSVRGV	22.2	13.3	2.9	4.6 0.2
20E2A-4-F3-IGFR	MQSGFSFYDAIDRLVGRIGER	21.2	19.0	4.4	4.4 0.2
20E2A-3-B2-IGFR	VGSSSFYEAIERLVQGLGRH	20.6	19.3	4.6	4.2 0.2
20E2A-4-G8-IGFR	LSWAAGFYEAIDQLVRSGGHR	18.7	14.7	3.8	3.9 0.3
20E2A-3-D10-IGFR	QQVHAGFYEAIEELVGFGLG	20.9	10.8	2.7	3.9 0.3
20E2A-3-A12-IGFR	MMVVDGFYDAIHQLVVAQSLG	20.6	6.9	1.8	3.9 0.3
20E2A-3-A11-IGFR	LSVALSFYDALGQLVAGEGRW	16.1	4.3	1.1	3.9 0.3
20E2A-4-H1-IGFR	SGSNLGFYDALRQLVGTADGS	17.8	9.7	2.6	3.7 0.3
20E2A-4-F7-IGFR	PSGFLSFYEAIDQLVHGVWVF	20.8	14.5	4.1	3.5 0.3
20E2A-3-D7-IGFR	AFTPTSFYDAIEQLVQQLSPR	19.5	17.9	5.3	3.4 0.3
20E2A-3-A9-IGFR	VSSLRSFYDALDELVRPFQQ	22.0	18.3	5.6	3.3 0.3
20E2A-3-A10-IGFR	VSMFQSFYDALKQLVRGISEG	24.7	10.5	3.2	3.3 0.3
20E2A-3-B11-IGFR	IGVSRGFYDAIDKLVRDRGSP	26.3	15.4	4.8	3.2 0.3
20E2A-3-D12-IGFR	GRSLLSFYDLIDQLVQAGNGG	15.8	10.7	3.4	3.2 0.3
20E2A-4-H11-IGFR	GQRAQSFYEAIALARLVCEGRCT	13.9	9.0	2.8	3.2 0.3
20E2A-4-H5-IGFR	CRFQGSFYDAIDLLVLGVRTC	22.8	17.5	5.7	3.1 0.3
20E2A-4-E11-IGFR	RWAFQSFYDAIDHLVNHREGH	20.1	16.6	5.5	3.0 0.3
	LPSSSGFYNAIQQLVCGHRGC	21.0	12.6	4.2	3.0 0.3

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FIG. 2M-1

Clone Design	Sequence	Ratios over Background				Comparisons	
		E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	
20E2A-3-C2-IR	XXXXXXFYDAIDQLVXXXXXX	23.4	20.9	15.4	1.4	--	0.7
20E2A-3-B1-IR	RPQGGTFYDMIKQLVLGSGWG	22.1	21.6	15.6	1.4	--	0.7
20E2A-3-A8-IR	WSAFADFYDAIQHLVAGEVGA	12.3	4.8	2.7	1.8	--	0.6
20E2A-4-G2-IR	SDGRDGFYDAIQQLVRSAFGD	18.9	13.8	7.9	1.8	--	0.6
20E2A-3-A9-IR	IRSVFSFYDAIDQLVKGKGS	23.3	20.3	11.3	1.8	--	0.6
20E2A-3-D3-IR	GGVSLTFYEAIEQLVRRGFDA	24.4	24.5	13.5	1.8	--	0.6
20E2A-3-A11-IR	AAQAFSFDLINQLVASKPSE	13.5	4.6	2.7	1.7	--	0.6
20E2A-3-B4-IR	QSGACGFYDAINQLVLGSIC	21.4	15.3	8.9	1.7	--	0.6
20E2A-4-E10-IR	GGIVFSFYEAIDQLVRRNGAG	22.3	19.0	11.3	1.7	--	0.6
20E2A-3-D2-IR	IYTGQGFYDAIEQLVRRGSTP	22.5	19.0	11.2	1.7	--	0.6
20E2A-4-F1-IR	KSPALSFYDAIEQLVGSQVVR	14.5	6.2	3.9	1.6	--	0.6
20E2A-3-D1-IR	ISPPWTFYDAIDQLVGSQDGR	16.5	6.6	4.0	1.6	--	0.6
20E2A-3-D11-IR	GSRFRGFYDAIDQLVRRQGLE	20.2	14.4	8.9	1.6	--	0.6
20E2A-3-C3-IR	GVAGGTFYDAIEQLVRRQFGGS	23.9	22.5	14.2	1.6	--	0.6
20E2A-3-C12-IR	RPLRWSFYDALDQLVGSALGG	21.3	23.0	14.4	1.6	--	0.6
20E2A-3-A2-IR	MQGRGGFYDAIADLVGSHVRG	18.9	11.6	7.5	1.5	--	0.6
20E2A-3-C7-IR	TSQGLSFYDAINQLVAGWG	21.6	15.1	6.9	2.2	--	0.5
20E2A-3-C10-IR	SGGTTFYDAINQLVIRGYN	18.1	18.0	9.1	2.0	--	0.5
20E2A-3-D9-IR	GGALDPFYDAIYQLVIRGSSG	21.8	21.6	8.4	2.6	--	0.4
20E2A-3-B5-IR	KQRGVTFYDLNQLVGSARG	24.3	18.1	7.4	2.5	--	0.4
20E2A-3-A6-IR	PRAPRSFYDAIHQLVGRQPG	17.8	19.1	7.6	2.5	--	0.4
20E2A-4-G12-IR	PCSDQFYDALSQLVGIRVCP	9.0	9.3	2.6	3.6	--	0.3
	SYGYQSFYDAIEELVRRPPAR						

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FIG. 2L-3

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
20E2A-3-B3-IR	XXXXXXXXFYDAIDQLVXXXXXXXX	22.4	18.6	15.9	1.2
20E2A-4-H10-IR	RSSCQSFYDAIERLVGGTCG	24.8	24.5	21.2	1.2
20E2A-4-F8-IR	VSRKFSFYDAIQQLVRGDAGV	4.3	2.2	2.1	1.1
20E2A-4-H5-IR	FORTWSFYDAINQLVMEGSGD	21.3	18.3	16.5	1.1
20E2A-4-G1-IR	RGSATTFYDAINQLVQDGGW	21.4	18.3	16.0	1.1
20E2A-4-F2-IR	AQPCVSFYDAIEQLVTGRSCM	20.1	20.5	18.2	1.1
20E2A-4-H7-IR	GGDGPFFYDWIEQLVRAGSEA	22.6	21.2	18.6	1.1
20E2A-3-D5-IR	LDLCASFYDAIEQLVGVKFCG	22.7	21.3	18.9	1.1
20E2A-3-A1-IR	WLACQSFYDAIDQLVGGECN	23.8	21.7	19.9	1.1
20E2A-4-H9-IR	EVNALSFYDAIDQLVRGGLGG	24.0	22.5	20.8	1.1
20E2A-3-D7-IR	RLQPRTFYEAIDQLIGGVLEG	24.1	23.5	21.0	1.1
20E2A-4-E5-IR	SGAHRTFYDAIQELVGMGSGK	24.1	23.5	20.9	1.1
20E2A-4-E11-IR	NMQSLTFYDAIAQLVLGRSGG	22.5	14.6	11.7	1.3
20E2A-3-A4-IR	RAVGATFYDQINQLVRKDDGY	20.2	17.5	13.4	1.3
20E2A-4-G8-IR	SQCRGGFYDAIYQLVTGVNCI	21.7	18.0	13.8	1.3
20E2A-3-B7-IR	DRLAFSFYDAIDQLVHCCGHG	21.1	21.4	16.9	1.3
20E2A-4-G11-IR	GNSVLSFYDAIAQLVGGQSI	22.9	23.1	17.7	1.3
20E2A-3-C5-IR	RSGPMSFYDAIEQLVLGRLHP	24.2	24.3	19.0	1.3
20E2A-4-E8-IR	VSGCRTFYDAIDQLVSGQACG	17.1	11.5	9.4	1.2
20E2A-4-H2-IR	AQPRTFYDAIEQLIHGKGM	21.6	13.7	11.6	1.2
20E2A-4-H4-IR	CAQPFYDAIDRLVTGRCLV	21.3	19.6	16.3	1.2
20E2A-3-D4-IR	PDECQSFYCAIDRLVTGKGR	23.2	22.2	18.0	1.2
20E2A-4-F5-IR	QRRARDFYEAIQQLVGGVAGL	12.2	5.7	3.8	1.5
20E2A-3-B10-IR	PLVRGTFYDAIKQLVMGSSD	14.9	5.9	3.9	1.5
20E2A-3-B9-IR	VGTAWTFYDAIQQLVGRSPEG	15.5	11.0	7.2	1.5
20E2A-3-D10-IR	PRGQASFYDMIEQLVGSADWN	22.2	19.1	12.8	1.5
20E2A-3-D6-IR	DGRVMSFYDALEQLVQGEGP	21.8	19.3	13.0	1.5
20E2A-4-G9-IR	RFVRSFYDAIEQLILAPNLG	21.3	19.9	13.3	1.5
20E2A-4-E1-IR	KVGRGSFYDAIRELVGQGHV	23.1	20.7	13.6	1.5
20E2A-4-F12-IR	PAIGTFYDAIRQLVWFQAD	17.5	17.1	12.1	1.4
20E2A-4-G3-IR	ALPGRSFYDAIAQLVGPDWGA	21.6	19.4	14.1	1.4

FIG. 2L-2

Cl ne Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFSR	IR	IGFSR	IR	IR/IGFSR
Parental	XXXXXXXXFYDAIDQLVXXXXXX	--	--	--	--	--	--
20E2A-4-F9-IR	FYDAIDQLVRSARAGGTRD	30.6	15.1	4.2	3.6	0.3	0.3
20E2A-4-E2-IR	PPWGARFYDAIEQLVFDNLCC	19.9	1.2	13.9	0.1	11.5	11.5
20E2A-3-B6-IR	IGRVRSFYDAIDKLFQSDWER	13.9	1.3	9.7	0.1	7.6	7.6
20E2A-3-A7-IR	RDAGSSFYDAIDQLVCLTYFC	14.5	1.6	6.1	0.3	3.8	3.8
20E2A-4-F7-IR	MPMGLNFYDGIQLVREWGDD	18.6	4.2	15.2	0.3	3.6	3.6
20E2A-3-C9-IR	TISAHTFYDAIYQLIEGIDPL	20.1	10.6	15.8	0.7	1.5	1.5
20E2A-3-C11-IR	SPWGRAFYDALDQLMGGAERG	24.4	16.4	23.8	0.7	1.4	1.4
20E2A-4-G7-IR	LSPPRDFYDAIQQLVRDGGWG	14.8	5.9	6.6	0.9	1.1	1.1
20E2A-4-H11-IR	HGVPRTFYDAIDQLVWGIEVG	17.2	8.3	8.9	0.9	1.1	1.1
20E2A-4-E9-IR	GGTDQLFYGAIDQLVGGTWR	25.4	26.9	25.6	1.1	1.0	1.0
20E2A-4-F4-IR	LSVHQSFYDAINELEIFSGLEA	4.7	2.1	2.1	1.0	1.0	1.0
20E2A-3-A3-IR	GDARDPFYDAMEQLVYGELEG	12.6	5.6	5.7	1.0	1.0	1.0
20E2A-4-E4-IR	VASPRSFYEAIAQLVFNLGQE	21.0	11.9	12.1	1.0	1.0	1.0
20E2A-4-G5-IR	RKPCQTFYDCILDLVVTDDV	21.0	16.0	16.1	1.0	1.0	1.0
20E2A-4-H1-IR	LLSRWTFYDAIEQLVGGGADG	22.1	19.5	18.8	1.0	1.0	1.0
20E2A-3-D8-IR	PAGCQGFYEAIEQLVTGCECG	24.4	20.4	21.2	1.0	1.0	1.0
20E2A-4-F11-IR	AVFPRTFYEAIDQLVGVSLIG	22.3	20.8	20.3	1.0	1.0	1.0
20E2A-3-C1-IR	APIPFSFYDAIVQLVMQGDHE	23.2	21.0	20.4	1.0	1.0	1.0
20E2A-3-B2-IR	QCNPRTFYEAIAQLVTGCDVS	23.4	22.3	22.5	1.0	1.0	1.0
20E2A-4-G10-IR	VSTSGSFYDAIQQLLEDWSGW	24.6	22.5	22.8	1.0	1.0	1.0
20E2A-3-A5-IR	HHSAFSFYDAIAQLVGVPEEE	21.5	22.9	22.2	1.0	1.0	1.0
20E2A-4-H3-IR	FTYVHSFYDAIEQLVRGEGGG	24.6	23.4	22.6	1.0	1.0	1.0
20E2A-3-C4-IR	QGNQNFYDAIDQLCFGLGG	24.5	24.1	25.1	1.0	1.0	1.0
20E2A-4-E3-IR	SSEGWTFYDAIDQLVGRERGW	25.2	24.4	24.7	1.0	1.0	1.0
20E2A-4-E7-IR	PDGCATFYHAIQQLVTGFPCV	18.5	15.7	13.6	1.2	0.9	0.9
	RGPPMTFYDAIAQLVAQASDG	17.8	16.6	14.4	1.2	0.9	0.9

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FIG. 2L-1

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF ₃ R	IGFR/IR	IR/IGFR
B6C-3-C4-IGFR	EHWNTVDPPFYHKLSELLRESGA	--	--	--	--
B6C-3-F5-IGFR	EHWNTVDPPFYHKLSELLRESGA	21.0	23.1	--	--
B6C-3-D4-IGFR	EHWNTVDPPFYHKLSELLRESGA	26.5	22.6	--	--
B6C-3-A3-IGFR	EHWNTVDPPFYHKLSELLRESGA	26.6	22.1	--	--
B6C-3-C9-IGFR	EHWNTVDPPFYHKLSELLRESGA	29.7	20.8	--	--
B6C-3-C8-IGFR	EHWNTVDPPFYHKLSELLRESGA	29.6	20.5	--	--
B6C-3-A8-IGFR	EHWNTVDPPFYHKLSELLRESGA	30.5	19.9	--	--
B6C-3-A2-IGFR	EHWNTVDPPFYHKLSELLRESGA	29.7	19.7	--	--
B6C-3-A9-IGFR	EHWNTVDPPFYHKLSELLRESGA	33.6	19.0	--	--
B6C-3-C10-IGFR	EHWNTVDPPFYHKLSELLRESGA	33.0	18.3	--	--
B6C-3-F7-IGFR	EHWNTVDPPFYHKLSELLRESGA	30.0	17.7	--	--
B6C-3-F10-IGFR	EHWNTVDPPFYHKLSELLRESGA	27.3	17.6	--	--
B6C-3-C12-IGFR	EHWNTVDPPFYHKLSELLRESGA	27.4	17.4	--	--
B6C-3-E11-IGFR	EHWNTVDPPFYHKLSELLRESGA	28.4	17.3	--	--
B6C-3-F8-IGFR	EHWNTVDPPFYHKLSELLRESGA	28.0	17.2	--	--
B6C-3-F2-IGFR	EHWNTVDPPFYHKLSELLRESGA	25.4	16.0	--	--
B6C-3-B6-IGFR	EHWNTVDPPFYHKLSELLRESGA	30.8	15.1	--	--
B6C-3-D5-IGFR	EHWNTVDPPFYHKLSELLRESGA	2.9	14.7	--	--
B6C-3-A4-IGFR	EHWNTVDPPFYHKLSELLRESGA	2.4	14.3	--	--
B6C-3-D3-IGFR	EHWNTVDPPFYHKLSELLRESGA	22.6	13.9	--	--
B6C-3-F9-IGFR	EHWNTVDPPFYHKLSELLRESGA	28.0	13.8	--	--
B6C-3-A7-IGFR	EHWNTVDPPFYHKLSELLRESGA	24.8	13.6	--	--
B6C-3-H10-IGFR	EHWNTVDPPFYHKLSELLRESGA	14.4	12.8	--	--
B6C-3-H11-IGFR	EHWNTVDPPFYHKLSELLRESGA	17.1	11.1	--	--
B6C-3-A1E-IGFR	EHWNTVDPPFYHKLSELLRESGA	2.3	11.0	--	--
B6C-3-E12-IGFR	EHWNTVDPPFYHKLSELLRESGA	23.3	10.5	--	--
B6C-3-H12-IGFR	EHWNTVDPPFYHKLSELLRESGA	2.7	10.1	--	--
B6C-3-G1-IGFR	EHWNTVDPPFYHKLSELLRESGA	2.3	9.9	--	--
		--	--	--	--

FIG. 2K

		Ratios over Background		Comparisons	
Clone Design	Sequence	E-Tag	IGFsR	IR	IGFR/IR
B6C-4-F4-IR	EHWNTVDPPFYHKLSELLRESG	36.3	36.9	34.3	1.1
B6C-3-D2-IR	EHWNTVDPPFYQKLFELLRESG	8.5	10.3	9.0	1.2
B6C-3-A1-IR	EHWNTVDPPFYH*LAELLRESG	26.5	30.7	16.8	1.8
B6C-3-B5-IR	EHWNTVDPPFYH*INELLRESG	33.4	33.0	15.0	2.2
B6C-3-A4-IR	EHWNTVDPPFYRLQELLRESG	33.6	31.8	13.5	2.4

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0.9
0.5
0.5
0.4

FIG. 2J-3

Cl ne Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
B6C-3-C11-IR	EHWNTVDPPFYHKLSELLRESG	33.4	35.0	36.7	1.0 1.0
B6C-3-C4-IR	EHWNTVDPPFYD*ISELLRESG	34.5	35.2	36.8	1.0 1.0
B6C-3-B6-IR	EHWNTVDPPFYHLLQELLRESG	34.2	36.1	36.9	1.0 1.0
B6C-4-E5-IR	EHWNTVDPPFYHMLQELLRESG	35.5	35.8	36.9	1.0 1.0
B6C-4-H12-IR	EHWNTVDPPFYH*MSDLLRESG	37.0	36.2	37.1	1.0 1.0
B6C-4-F9-IR	EHWNTVDPPFYHQLQELLRESG	36.1	36.2	37.3	1.0 1.0
B6C-3-A6-IR	EHWNTVDSFYLGQLQELLRESG	35.8	38.0	37.4	1.0 1.0
B6C-3-D1-IR	EHWNTVDPPFYQGLSELLRESG	36.2	37.8	37.5	1.0 1.0
B6C-3-D4-IR	EHWNTVDPPFYQALQELLRESG	35.9	36.4	37.5	1.0 1.0
B6C-3-C1-IR	EHWNTVDPPFYIMLQELLRESG	36.8	36.6	37.9	1.0 1.0
B6C-4-G5-IR	EHWNTVDPPFYKMQDLLRESG	36.5	37.9	38.0	1.0 1.0
B6C-3-A8-IR	EHWNTVDPPFYQKQLQELLRESG	34.4	37.0	38.1	1.0 1.0
B6C-4-H1-IR	EHWNTIDPPFYHQISELLRESG	36.8	36.5	38.3	1.0 1.0
B6C-3-D10-IR	EHWNTVDPPFYH*MTPELLRESG	37.0	37.0	38.5	1.0 1.0
B6C-3-D12-IR	EHWNTVDPPFYHMSQELLRESG	36.2	37.0	38.7	1.0 1.0
B6C-3-B9-IR	EHWNTVDPPFYQGLFELLRESG	36.3	37.3	38.8	1.0 1.0
B6C-4-H7-IR	EHWNTVDPPFYAKLQELLRESG	37.5	38.0	39.1	1.0 1.0
B6C-3-D11-IR	EHWNTVDPPFYH*MRELLRESG	36.1	37.5	39.2	1.0 1.0
B6C-4-F10-IR	EHWNTVDPPFYHGL*ELLRESG	37.9	38.6	39.3	1.0 1.0
B6C-4-G8-IR	EHWNTVDPPFYLGQLQELLRESG	35.9	38.0	39.7	1.0 1.0
B6C-3-A9-IR	EH*NTVDPPFYD*IAELLRESG	36.5	38.8	39.9	1.0 1.0
B6C-3-A7-IR	EHWNTVDFAFYHGLQELLRESG	38.1	39.4	40.2	1.0 1.0
B6C-4-F12-IR	EHWNTVDPPFYQGLIELLRESG	38.0	38.4	40.2	1.0 1.0
B6C-4-G9-IR	EHWNTVDPPFYN*MRELLRESG	37.5	39.4	40.5	1.0 1.0
B6C-4-H8-IR	EHWNTVDPPFYQGLDLLRESG	38.5	40.0	40.8	1.0 1.0
B6C-3-B10-IR	EHWNTVDPPFYQKQLQELLRESG	39.3	40.3	40.9	1.0 1.0
B6C-3-A10-IR	EHWNTVDPPFYHGLQELLRESG	38.4	40.9	41.6	1.0 1.0
B6C-3-A3-IR	EHWNTVDPPFYH*MSPELLRESG	39.2	40.0	41.7	1.0 1.0
B6C-3-A5-IR	EHWNTVDPPFYAGLQALLRESG	38.2	40.4	41.9	1.0 1.0
B6C-3-C3-IR	EHWNTVDPPFYHMLQKLLRESG	34.5	34.6	32.0	1.1 0.9

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FIG. 2J-2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
B6C-3-C7-IR	EHWNTVDPPFYHKLSELLRESG	31.7	2.1	20.0	0.1 9.4
B6C-4-F2-IR	EHWNTVDPPFYFTLFE*LRRESG	8.2	1.8	4.3	0.4 2.4
B6C-3-A2-IR	EHWNTVDPPFYQNLWEWLRRESG	34.9	18.1	36.0	0.5 2.0
B6C-4-H11-IR	EHWNTVDPPFYHQLSEWLRRESG	37.1	28.2	38.6	0.7 1.4
B6C-4-H4-IR	EHWNTVDPPFYQQLYEWLRRESG	39.5	28.3	39.4	0.7 1.4
B6C-3-A11-IR	EHWNTVDPPFYHQLSEWLRRESG	25.4	25.9	34.2	0.8 1.3
B6C-3-D9-IR	EHWNTVDPPFYHQLSEWLRRESG	35.7	30.3	37.2	0.8 1.2
B6C-4-G4-IR	EHWNTVDPPFYHQLSEWLRRESG	35.3	31.0	38.4	0.8 1.2
B6C-3-C6-IR	EHWNTVDLFFYGLQELLRESG	33.3	33.9	35.9	0.9 1.1
B6C-3-D8-IR	EHWNTVDPPFYH*ISELLRESG	34.5	34.7	37.1	0.9 1.1
B6C-4-G7-IR	EHWNTVDPPFYQFAELLRESG	35.9	36.9	38.9	0.9 1.1
B6C-3-C8-IR	EH*NTVDPPFYEGLELLRESG	35.6	37.2	39.6	0.9 1.1
B6C-3-D6-IR	EH*NTVDPPFYQGLFELLRESG	37.6	37.6	40.2	0.9 1.1
B6C-3-C10-IR	EHWNTVDPPFYQXFSPELLRESG	35.3	36.4	40.6	0.9 1.1
B6C-3-B3-IR	EHWNTVDPPFYGLQELLRESG	38.3	38.7	40.8	0.9 1.1
B6C-3-B1-IR	EHWNTVDPPFYQALFELLRESG	37.8	38.9	41.2	0.9 1.1
B6C-4-F6-IR	EHWNTVDPPFYD*MRNLLRESG	35.8	36.8	38.7	1.0 1.1
B6C-3-B11-IR	EHWNTVDPPFYNLQELLRESG	36.3	37.0	38.8	1.0 1.1
B6C-3-B8-IR	EHWNTVDPPFYDGLRQLRESG	37.2	39.2	41.2	1.0 1.1
B6C-3-C12-IR	EHWNTVDPPFYGKIQELLRESG	28.3	28.7	28.9	1.0 1.0
B6C-3-C2-IR	EHWNTVDPPFYQQLFELLRESG	34.1	34.7	33.8	1.0 1.0
B6C-3-D5-IR	EHWNTVDPPFYMLQLLRESG	33.9	35.3	34.1	1.0 1.0
B6C-4-F7-IR	EH*NTVDPPFYHKLVELLRESG	34.9	34.7	34.2	1.0 1.0
B6C-4-H2-IR	EHWNTVDPPFYH*MSNLLRESG	35.4	35.8	35.8	1.0 1.0
B6C-3-B12-IR	EHWNTVDPPFY*MSPELLRESG	33.6	35.2	36.0	1.0 1.0
B6C-3-A12-IR	EHWNTVDPPFYQLLFFELLRESG	33.1	37.0	36.2	1.0 1.0
B6C-4-E9-IR	EHWNTVDPPFYQRMFELLRESG	36.1	36.0	36.2	1.0 1.0
B6C-4-E8-IR	EHWNTVDPPFYQGLWELLRESG	34.2	35.0	36.6	1.0 1.0

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FIG. 2J-1

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Clon Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
B6H-4-D8-IGFR	OOUUUUUUJJJJDPFYHKLSELLXOO	--	--	--	--
B6H-4-D6-IGFR	GGVKA AAAERDDSDPFYHKLSELLFGS	15.1	16.4	--	--
B6H-4-E10-IGFR	CCEMVKTIEHGGNDPFYHKLSELVFG	12.6	15.6	--	--
B6H-4-F5-IGFR	GGAKVAVVVDHGDDPFYHKLSELLRGS	10.2	15.1	--	--
B6H-4-B2-IGFR	RGKTKMAAAGGNRDPFYHKLSELLFG	12.3	14.8	--	--
B6H-3-F3-IGFR	GGMATKIVTAPGHPDYHKLSELLFG	6.6	11.8	--	--
B6H-3-A2-IGFR	SGEGEMAMPGDDPFYHKLSELLGSA	8.2	11.6	--	--
B6H-3-H2-IGFR	GGMAEVVVVGPPRDPFYHKLSELVGG	10.9	9.9	--	--
B6H-4-A1-IGFR	GGEVKVMVADGSTDPFYHKLSELLGRT	5.9	9.6	--	--
B6H-3-H1-IGFR	SCVMVETVAGNRDPFYHKLSELVGGC	4.4	9.5	--	--
B6H-4-C2-IGFR	RRW*KVPGAADPFYHKLSELLGRSA	7.2	8.7	--	--
B6H-4-H9-IGFR	GGVEATEVEHADGDPFYHKLSELLRGA	6.7	8.6	--	--
B6H-4-B7-IGFR	RGVEVAVITHGPPDPFYHKLSELLRS	12.3	8.4	--	--
B6H-4-B3-IGFR	SGTVTVIAMSGTDDPFYHKLSELLRG	6.4	8.2	--	--
B6H-4-B4-IGFR	GRTAVVKEASPAHDPFYHKLSELLRGS	9.7	8.1	--	--
B6H-4-E1-IGFR	RGAINAAVGNRSDPFYHKLSELLRGS	4.4	7.8	--	--
B6H-3-C1-IGFR	GGMIKTAMEHDTDPFYHKLSELLRGG	5.2	7.4	--	--
B6H-4-A3-IGFR	GCAEVEEVAGAGHDPFYHKLSELV?SG	3.6	7.1	--	--
B6H-4-H10-IGFR	SSVVVVEVVDARRDPFYHKLSELLRGS	5.7	4.6	--	--
B6H-3-G1-IGFR	GRKKAVATMTDGGDPFYHKLSELLRGS	4.4	4.2	--	--
B6H-4-H10-IGFR	RGETEMAVADTDDDPFYHKLSELLRGS	4.4	3.2	--	--
B6H-3-G1-IGFR	GQRDPFYHKLSELMGRGA	2.4	2.9	--	--

FIG. 21-2

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Cl ne Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
B6H-3-F1-IGFR	OOUUUUUUJJJJDDPFYHKLSELLXOO	20.0	30.8	--	--	--
B6H-3-D4-IGFR	RRVAAVA?KDATGDPFYHKLSELLRSG	19.0	27.6	--	--	--
B6H-3-G3-IGFR	RSTMKEKIEGDGNDPFYHKLSELLKSG	14.2	25.2	--	--	--
B6H-3-F2-IGFR	GGAVIVTAARRGSDPFYHKLSELLVGRG	12.5	24.8	--	--	--
B6H-3-D1-IGFR	SREAVEVTMARGSDPFYHKLSELLVWGS	20.0	24.2	--	--	--
B6H-3-A3-IGFR	RSTTMVKAVPFPRDPFYHKLSELL*GG	14.2	22.8	--	--	--
B6H-3-A4-IGFR	GRTEVVVVGTTRDPFYHKLSELLASG	13.0	22.8	--	--	--
B6H-3-B4-IGFR	RMAGWQ*TSSSDPFYHKLSELLVSGS	10.2	22.8	--	--	--
B6H-4-D11-IGFR	SRKEVTEMVGGPSDPFYHKLSELLVSGR	14.0	22.5	--	--	--
B6H-3-G2-IGFR	RGTAKQRKSSDP*DPFYHKLSELLVSG	15.2	22.5	--	--	--
B6H-3-B2-IGFR	GGVAVVAAGRRDDPFYHKLSELLVSGR	14.5	21.9	--	--	--
B6H-3-E2-IGFR	SR.MAMVEVGNPGDPFYHKLSELLVSG	11.6	21.8	--	--	--
B6H-3-B1-IGFR	RRVTAVIEVDGADDPFYHKLSELLVSG	15.9	21.7	--	--	--
B6H-4-G3-IGFR	RSVIAN??G?NADPFYHKLSELLVSG	19.1	21.4	--	--	--
B6H-4-F9-IGFR	RGVVIETTKDPGADPFYHKLSELLVSGR	11.3	20.9	--	--	--
B6H-3-E3-IGFR	RRTVMEVTVGGRDDPFYHKLSELLVHRG	14.2	20.8	--	--	--
B6H-3-E1-IGFR	GRVVAAAVRPDDDPFYHKLSELLVAGR	20.0	20.6	--	--	--
B6H-4-F3-IGFR	RGVATVVVANHHSDPFYHKLSELLVIRG	12.5	20.3	--	--	--
B6H-3-D2-IGFR	RRKMATEIMRSDADPFYHKLSELLVGG	12.1	19.3	--	--	--
B6H-4-A9-IGFR	GGKTAVEVTSASDPFYHKLSELLVLRG	14.1	19.2	--	--	--
B6H-4-E6-IGFR	RREKKVKVTTTNDNPFYHKLSELLVFGG	12.5	19.2	--	--	--
B6H-4-C3-IGFR	SSATIMVAADRAADDPFYHKLSELLVWGS	23.6	18.9	--	--	--
B6H-3-C2-IGFR	RRMVEAAENHADDPFYHKLSELLVWRD	16.2	18.5	--	--	--
B6H-3-C4-IGFR	GRKMEIVAIRGAHDPFYHKLSELL*GR	16.8	17.2	--	--	--
B6H-3-C3-IGFR	CCIAMVEMAAGGDDPFYHKLSELLVSGR	14.6	17.1	--	--	--
B6H-3-B3-IGFR	RGAQSPDPFYHKLSELLAFGS	9.0	16.8	--	--	--
B6H-4-H3-IGFR	RKTAMVIGDASDPFYHKLSELLAFGS	10.1	16.6	--	--	--
B6H-3-H3-IGFR	GSVITKAMKADGDDPFYHKLSELL*GG	14.2	16.4	--	--	--

FIG. 21-1

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
B6Hβ-3-H12-IR	OOUUUUUUUUJJJDDPFYHKLSELLXXOO	--	--	--	--
B6Hβ-3-G10-IR	RGEAKEAKIGSAGDPFYHKLSELMQGSR	33.6	32.0	29.5	1.1 0.9
B6Hβ-3-F10-IR	GCEVVVMANSSADPFYHKLSELCQGSR	30.1	34.3	30.5	1.1 0.9
B6Hβ-3-D5-IR	GCAAVVTTGGDNDPFYHKLSELLQGCR	37.1	35.3	32.4	1.1 0.9
B6Hβ-3-B12-IR	SRTGERQVVGSHADPFYHKLSELLSS	39.9	38.9	35.5	1.1 0.9
B6Hβ-3-D2-IR	GCKEVVETAHADDPFYHKLSELLQGCR	39.5	40.0	37.1	1.1 0.9 43/200
B6Hβ-3-D1-IR	RRITIKVKAGDDDDPFYHKLSELLWGG	40.4	41.5	39.1	1.1 0.9
B6Hβ-3-G6-IR	WCDQKETVVSNSDDPFYHKLSELVGCS	41.1	44.6	36.6	1.2 0.8
B6Hβ-3-A7-IR	RCEIITIGDGRAGDPFYHKLSELLQGC	34.3	36.4	24.1	1.5 0.7
B6Hβ-3-B10-IR	CSVVMTTEKNDRDDPFYHKLSELLQGC	38.1	30.9	18.4	1.7 0.6
B6Hβ-3-B9-IR	GGEARRRQQVGTANDPFYHKLSELAFGGR	32.3	36.5	22.8	1.6 0.6
B6Hβ-3-D6-IR	GCAVTAITINGTSDPFYHKLSELCQGS	38.6	38.5	20.8	1.9 0.5
B6Hβ-3-C7-IR	GSKVKAMAVGTSDDPFYHKLSELVQGR	35.9	36.0	15.6	2.3 0.4
	RCKGIKAHSDNDPFYHKLSELCQGG	38.3	38.0	6.6	5.8 0.2

FIG. 2H-4

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
B6H β -3-D7-IR	OOUUUUUUJJJDDPFYHKLSELXXOO	39.5	27.1	26.3	1.0
B6H β -3-B2-IR	RGVEMKAIVGTPNDPFYHKLSELSSGS	34.3	34.4	35.4	1.0
B6H β -3-G5-IR	CSAVKMAEAGDPSDPFYHKLSELCCQGS	35.3	35.0	35.6	1.0
B6H β -3-H1-IR	RGGDGDPPFYHKLSELQSS	36.8	35.4	36.5	1.0
B6H β -3-A5-IR	WICKRQTHDPPFYHKLSELACGR	34.9	35.5	35.9	1.0
B6H β -3-H11-IR	SSKVVKATVGTDPDPFYHKLSELQGS	37.7	36.4	37.6	1.0
B6H β -3-C2-IR	GCAAIATGNDNDPFYHKLSELQGR	37.4	36.5	37.2	1.0
B6H β -3-C8-IR	GCAAEKEVAGTARDPFYHKLSELMQSS	37.0	37.7	39.5	1.0
B6H β -3-A11-IR	CSVAVGDSGDPFYHKLSELQGR	40.4	38.2	39.1	1.0
B6H β -3-D8-IR	WQNKQIIGTPDDPFYHKLSELLEGS	35.4	38.3	39.5	1.0
B6H β -3-B7-IR	RSAAKAVIGSPNDPFYHKLSELIQGG	37.8	39.0	39.4	1.0
B6H β -3-A12-IR	WLCDRDGRDEQWDPFYHKLSELVSCGR	33.5	39.4	41.3	1.0
B6H β -3-B4-IR	GSVAAAKKTGSSDDPFYHKLSELQGS	39.0	39.8	41.1	1.0
B6H β -3-A4-IR	GCAVTTMTMRSADPFYHKLSELCCQGR	40.1	40.4	41.1	1.0
B6H β -3-E12-IR	GCKVDDE*ARSSDPFYHKLSELKGR	35.8	40.7	40.7	1.0
B6H β -3-B8-IR	GCKAVVEVKDHGDDPFYHKLSELQGC	40.8	40.7	39.5	1.0
B6H β -3-C5-IR	CSTVTVSGSDDPFYHKLSELQGC	40.7	40.9	42.6	1.0
B6H β -3-A2-IR	RSVTAKVEVGSDDPFYHKLSELQGS	41.1	41.4	41.9	1.0
B6H β -3-A8-IR	GSRRQKIEVGTNDPFYHKLSELQGG	40.0	41.9	41.6	1.0
B6H β -3-C12-IR	LCDEKQRTVGTNDPFYHKLSELQGR	39.8	42.0	41.3	1.0
B6H β -3-B11-IR	SCMVEGPNDDPFYHKLSELQGR	40.7	42.6	43.3	1.0
B6H β -3-C3-IR	GGAUVVAMGNDDPFYHKLSELQGG	43.0	42.7	44.0	1.0
B6H β -3-B5-IR	GGVIKAMKAGPDDPFYHKLSELQGS	42.7	43.9	45.4	1.0
B6H β -3-C4-IR	GCIIAEKVVGPPDDPFYHKLSELDCG	41.1	42.3	36.6	0.9
B6H β -3-G7-IR	GCEKVAVAGNAGDPFYHKLSELQGC	4.1	2.4	2.1	0.9
B6H β -3-C11-IR	GSVMTVTTEMAGADDPFYHKLSELQGR	29.2	30.6	28.2	1.1

FIG. 2H-3

Cl ne Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	IR/IGFR
B6H α -1-A6-IR	OOUUUUUUJJJJDDPFYHKLSELLXOO	49.7	34.5	4.0	8.6	0.1	0.1
B6H α -2-C5-IR	GCKMEETETGTSDDPFYHKLSELLCSGG	42.6	34.2	3.3	10.4	0.1	0.1
B6H α -2-C4-IR	RGEVATMEVPAGGDPFYHKLSELLWGS	20.7	9.9	0.9	11.0	0.1	0.1
B6H α -2-C9-IR	RCGRW*AEAGAGDDPFYHKLSELLVCG	47.4	32.6	2.8	11.6	0.1	0.1
B6H α -4-H8-IR	RCMVETIAVSGDDPFYHKLSELLCQGG	36.3	28.1	2.4	11.7	0.1	0.1
B6H α -3-F11-IR	WWQKSGDGASASDPFYHKLSELLIWS	49.5	18.7	1.6	11.7	0.1	0.1
B6H α -3-E9-IR	RGKKEVLVGSTDPFYHKLSELLQGS	44.6	24.2	1.7	14.2	0.1	0.1
B6H α -1-A2-IR	RCEEKQAEVGPSSDPFYHKLSELLGCR	22.3	14.6	1.0	14.6	0.1	0.1
B6H α -1-B5-IR	RGCNDDGGKWSDDPFYHKLSELLICGG	41.5	20.5	1.0	20.5	0.0	0.0
B6H β -3-G4-IR	CCTTEMVMDARDDPFYHKLSELLVTGG	36.4	28.4	36.0	0.8	1.3	1.3
B6H β -3-A10-IR	GCKKVEAKKGNADPFYHKLSELLQGC	36.7	27.9	34.7	0.8	1.2	1.2
B6H β -3-D9-IR	RSMMAKAIVGGPGDPFYHKLSELLQFGR	34.9	32.1	35.6	0.9	1.1	1.1
B6H β -3-A3-IR	CGGAVPDGDDPFYHKLSELLMOGC	36.3	33.7	37.3	0.9	1.1	1.1
B6H β -3-G1-IR	GCEEVEAETTGRDPPFYHKLSELLQGC	34.7	33.7	35.9	0.9	1.1	1.1
B6H β -3-B3-IR	GCAEIEIAAGGGDPFYHKLSELLQGC	35.9	35.1	37.4	0.9	1.1	1.1
B6H β -3-G11-IR	GCAEVKAVKGAGDDPFYHKLSELLQGC	37.6	36.2	39.0	0.9	1.1	1.1
B6H β -3-F5-IR	GCAAVETTTNGRNDPFYHKLSELLQGC	39.2	37.2	41.0	0.9	1.1	1.1
B6H β -3-A1-IR	GCEVTGRAGDPFYHKLSELLQGC	41.3	38.0	43.0	0.9	1.1	1.1
B6H β -3-H3-IR	GCMVEATEGRRHDPFYHKLSELLQGC	39.0	38.3	40.7	0.9	1.1	1.1
B6H β -3-D3-IR	GQCAMEEIIRGANDPFYHKLSELLCEGG	38.8	38.4	41.3	0.9	1.1	1.1
B6H β -3-C9-IR	GCAEIVIEGGDDSDPFYHKLSELLQGC	36.7	39.2	41.5	0.9	1.1	1.1
B6H β -3-F1-IR	PQCSSIKAEGSGDDPFYHKLSELLVGC	41.5	40.0	42.2	0.9	1.1	1.1
B6H β -3-C6-IR	GCAAVVAEASGDDPFYHKLSELLQGC	39.9	40.3	42.7	0.9	1.1	1.1

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FIG. 2H-2

Clone Design	Sequence	Ratios over Background			Comparis ns		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	IR/IGFR
B6H α -3-F5-IR	OOUUUUUUJJJDDFFYHKLSELXXOO	42.7	9.6	17.9	0.5	1.9	1.9
B6H α -2-D10-IR	GGAVAAAVVGSRADPPFYHKLSELVQGS	20.8	1.7	1.1	1.5	0.6	0.6
B6H α -3-F1-IR	SGGGQQRKAIAATSDPPFYHKLSELLGG	22.5	2.4	1.3	1.8	0.5	0.5
B6H α -3-E6-IR	CSMAVAEAGDDDDPPFYHKLSELVQGS	18.2	2.3	1.2	1.9	0.5	0.5
B6H α -1-B8-IR	CGAKMTGTPNDPPFYHKLSELLORG	44.6	5.2	2.1	2.5	0.4	0.4
B6H α -2-D5-IR	CCVEAEAVGRRGDPFYHKLSELTGCC	39.6	2.3	0.9	2.6	0.4	0.4
B6H α -1-B3-IR	SRVVTMVIKRGSPDPFYHKLSELVQGR	33.1	3.2	1.1	2.9	0.3	0.3
B6H α -3-E5-IR	GCITAENGAGDPFYHKLSELGGCS	28.8	2.9	1.0	2.9	0.3	0.3
B6H α -4-H9-IR	RCGDEEGWQENRRDDPPFYHKLSELFGGC	17.4	6.4	2.1	3.0	0.3	0.3
B6H α -2-D8-IR	GCEVIAAEGRRDDPPFYHKLSELVQGG	19.3	3.0	1.0	3.0	0.3	0.3
B6H α -3-E4-IR	SSETAKMVTGTRDDPPFYHKLSELVQGS	43.1	8.7	2.8	3.1	0.3	0.3
B6H α -3-F7-IR	WLCDGGWKQRRPPGDPFYHKLSELIDCG	41.5	3.1	1.0	3.1	0.3	0.3
B6H α -1-A3-IR	SRVAATKEKRPSDDPPFYHKLSELLQGS	37.4	2.6	0.8	3.3	0.3	0.3
B6H α -4-H10-IR	SRAKVEAEMPDSGDPFYHKLSELLASG	50.5	29.5	8.6	3.4	0.3	0.3
B6H α -3-F6-IR	GGAAKTVVVGSPDDPPFYHKLSELLQGS	48.9	19.7	5.7	3.5	0.3	0.3
B6H α -3-F3-IR	CGVGEQMEVTDGDDPPFYHKLSELLMSC	18.1	15.6	4.3	3.6	0.3	0.3
B6H α -4-G8-IR	SGETATIEGPSNDPPFYHKLSELLWGS	32.3	6.1	1.7	3.6	0.3	0.3
B6H α -2-D1-IR	GGTKAVAKVGTDRDDPPFYHKLSELLQGS	11.7	5.4	1.3	4.2	0.2	0.2
B6H α -3-E7-IR	GCEVIVEEGDSADPPFYHKLSELVQGS	47.0	5.6	1.3	4.3	0.2	0.2
B6H α -2-D6-IR	GCAVVEEAERSRGDPFYHKLSELLQGC	33.5	4.4	1.0	4.4	0.2	0.2
B6H α -3-F10-IR	GRTMAVMAAGPDDPPFYHKLSELVQGG	47.2	8.8	1.9	4.6	0.2	0.2
B6H α -3-E8-IR	GCVVEWQKWHGASDPPFYHKLSELGGCS	47.6	5.3	1.1	4.8	0.2	0.2
B6H α -2-C10-IR	RGKTAAVIVGRPADPPFYHKLSELLQGG	46.9	5.8	1.1	5.3	0.2	0.2
B6H α -2-C7-IR	SGAKVIVVTGSDGDPFYHKLSELLQGS	45.1	6.7	1.0	6.7	0.1	0.1
	RGIVAMVEATEVGSDDHDPFYHKLSELVQGS						

FIG. 2H-1

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF ₃ R	IGFR/IR	IR/IGFR
B6L-4-G6-IGFR	AETPAQVGNRLMSVWPGEHWNNTVDPFYHKLSELLRESGA	--	--	--	--
B6L-4-G10-IGFR	AETPAQVGGDRMLMSVWPGEHWNNTVDPFYHKLSELLRESGA	7.3	22.1	--	--
B6L-4-G3-IGFR	AE?PAQVGNRLMSVWPGE?WNTVDPFYHKLSELL?ESGA	5.5	21.8	--	--
B6L-3-F10-IGFR	AET?AQVGWNG?WSVWPGEHWNNTVDPFYHKLSELLRESGA	5.8	18.1	--	--
B6L-4-D2-IGFR	AETPAQVQWNRRLMSVWPGEHWNNTVDPFYHKLSELLRESGA	6.7	17.4	--	--
B6L-3-H10-IGFR	AETRAQVGSNRVWSVWPGEHWNNTVDPFYHKLSELLRESGA	6.5	15.9	--	--
B6L-4-B12-IGFR	AETPAQVGNRLMSVQPGSDWNTVDPFYHKLSELLRESGA	5.9	15.2	--	--
B6L-3-A9-IGFR	DETPAQVGNRLMSDWPGEQWNTLDPFYHKLSELLRESGA	6.3	14.8	--	--
B6L-4-C4-IGFR	GETPAQVGNRLMSVWPAEHWSTVDPFYHKLSELLRESGA	5.2	14.8	--	--
B6L-4-E3-IGFR	AETAAQVGNRLQSVWPGEHWNNTVDPFYHKLSELLRESGA	5.4	11.9	--	--
B6L-4-A12-IGFR	AEAPDQVGNRLMSVWPGEHWNNTVDPFYHKLSELLRESGA	4.4	11.1	--	--
B6L-4-D5-IGFR	PETPAQVGNRLQSGWPGEHWNNTVDPFYHKLSELLRESGA	3.0	10.4	--	--
B6L-3-A10-IGFR	AQTPAQVGNRYRLSSVWPGEHWNNTVDPFYHKLSELLRESGA	4.7	10.3	--	--
B6L-3-B9-IGFR	AETPAQVGNRLMSVQPGEHWNNTVDPFYHKLSELLRQSDA	10.9	9.7	--	--
B6L-3-H9-IGFR	AETPAQVGNRLMSVWPGEH*NTVDPFYHKLSELLRESGA	2.9	9.1	--	--
B6L-4-A5-IGFR	AETPAQVGNRLQSVWPGEHWNNT?DPFYHKLSELLRESGA	4.5	8.8	--	--
B6L-3-H11-IGFR	AESPAQVGSNRRLQSVWSGEHWNNTVDPFYHKLSELLRESGA	2.4	8.0	--	--
B6L-3-G10-IGFR	AETPAQVQYRLSSVWPGEHGNNTVDPFYHKLSELLRESGA	4.2	7.5	--	--
B6L-4-D4-IGFR	AETPAQVGNRLMSVWPGEHWNNTIDPFY*KLSELLRESGA	2.5	7.4	--	--
B6L-4-F11-IGFR	DETPAHVGNRRLPQSAWPGERWNTVDPFYHKLSELLRESGA	2.4	6.8	--	--
B6L-4-F12-IGFR	AGTPAQVGNRLRSVQPDHWNNTVDPFYHKLSELLRESGA	2.1	6.4	--	--
B6L-4-E12-IGFR	AETPAQVGNRLMSVWPGEHWN?DPFYHKLSELLRESGA	2.7	5.9	--	--
B6L-4-E10-IGFR	AETPAQVGNRLQSVWPGEHWNNTVDPFYHKLSELLRESGA	1.9	3.9	--	--
B6L-3-G9-IGFR	AETPAQVGNRLMSVQPGEHWNNTVDPFYHKLSELLRESGA	1.8	3.6	--	--
	AETPAQVGNRLMSVWPGEHWNNTVDPFYHKLSELLRESGA	1.2	2.5	--	--

FIG. 2G

Cl ne	Parental/Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
B6L-4-C8-IR		AETPAQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGA	40.5	6.1	40.8	0.2	6.7	
B6L-4-B7-IR		ANILRLR?VGNRL*SVWPGEQWNTVDP?YQKLYELVRESGA	19.6	4.0	23.5	0.2	5.9	
B6L-3-H1-IR		AETPAQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGD	20.6	2.8	2.7	0.9	1.1	
B6L-4-E12-IR		AETPAHVC*TVGGLFGRVNTWNTVDPPFYAKLSELLRESGA	15.5	4.1	3.0	1.1	0.9	
B6L-4-D8-IR		GQSGSAWDGTSLSVWPBGDWNPNVDPPFYHKLSELLRESGA	36.0	9.4	8.5	1.1	0.9	
B6L-4-F7-IR		AEAPAQVGNLLOPGEHNTVDPPFYHKLSELLRESGD	37.8	24.6	20.6	1.2	0.9	
B6L-4-B11-IR		AETPAQVGNLMSVWPGEH*NTVDPPFYHKLSELLRESGA	5.5	2.0	1.6	1.3	0.8	
B6L-4-B12-IR		AETPAQVGNLMSVWPGEHNTVDPPFYHKLSELLRESGA	6.8	2.0	1.6	1.3	0.8	
B6L-4-B8-IR		AETPAQVGNLMSVWPGEHNTVDPPFYHKLSELLRESGA	36.4	18.7	14.2	1.3	0.8	
B6L-4-E8-IR		T*QGETPAQVSLMPGEHNTVDPPFYHKLSELLRESGA	35.6	11.4	8.6	1.3	0.8	
B6L-3-G6-IR		QGETPAQIGWNPLMSVWPGEHNTVDPPFYHKLSELLRESGA	7.6	2.5	1.8	1.4	0.7	38
B6L-3-G5-IR		VDTPAQVGNRLMSVGPGEHWYTDPPFYH*LSSELLRESGA	11.5	2.0	1.4	1.4	0.7	200
B6L-4-E10-IR		AETSAQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGA	14.8	3.2	2.2	1.5	0.7	
B6L-4-F10-IR		*NSPRVGNLMSVWPGEHNTVDPPFYHKLSELLRESGV	26.2	11.5	7.2	1.6	0.6	
B6L-3-F3-IR		AETPAQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGP	36.0	17.1	10.1	1.7	0.6	
B6L-4-A7-IR		ADTPAQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGA	11.6	3.4	1.9	1.8	0.6	
B6L-4-G8-IR		AGTPAQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGA	30.4	11.2	5.9	2.0	0.5	
B6L-4-F8-IR		D*QAWSVWPQHWNTVDPPFYHKLSELLRESGA	35.6	12.8	7.2	2.0	0.5	
B6L-4-G7-IR		AETLARVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGA	33.5	12.9	6.4	2.3	0.4	
B6L-3-F4-IR		AATRPQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGS	16.9	6.3	2.7	2.4	0.4	
B6L-3-H4-IR		LTTPAQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGA	20.6	4.9	2.0	2.5	0.4	
B6L-3-A6-IR		ADNPAQVGNRLMSVWPGEH*NTVDPPFYHKLSELLRESGA	22.4	6.3	3.2	3.2	0.3	
B6L-4-D7-IR		AETPAQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGA	14.3	4.8	1.4	3.4	0.3	
B6L-3-E2-IR		AETSVQVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGA	29.2	16.7	3.8	6.3	0.2	
		G*NSAHVGNRLMSVWPGEHNTVDPPFYHKLSELLRESGG						

FIG. 2F

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
20C-3-G3-IGFR	TFYSCLASLLTGTPQPNRGPWERC	33.1	32.3	1.2	27.0 <0.1
20C-4-C7-IGFR	FFYDCLAALLQGVARYHDLCAVEIT	35.3	28.0	1.3	21.8 <0.1
20C-3-F6-IGFR	DRDFCRFYERLTALVGGQVDGGWPC	33.5	26.1	1.9	14.1 0.1
20C-3-A1-IGFR	SSYGCDGFYLMFSLGLVASQEELEC	26.5	20.8	1.5	13.7 0.1
20C-3-A4-IGFR	QFYGCILDLSIGVPSFGWRRRCITA	17.7	8.8	1.2	7.6 0.1
20C-3-E4-IGFR	FFYRCLSRLLGGQLGSRGLSCIGD	37.7	7.7	1.3	6.0 0.2
20C-4-D11-IGFR	DLFYCMMQLATAGVGSIGGPVCG	33.3	17.2	6.1	2.8 0.4
20C-4-F7-IGFR	CDFYCALSRLSGQPRDRMPNYPGTS	31.2	28.9	17.0	1.7 0.6
20C-3-B2-IGFR	GSACDGFYACLHALVQGPGEW	37.7	35.2	30.9	1.1 0.9

FIG. 2E

Ratios over Background		Comparisons	
E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
--	--	--	--
43.7	30.8	3.0	10.3 0.1
46.3	39.9	3.1	12.9 0.1
48.6	39.9	2.4	16.6 0.1
18.5	28.9	4.3	6.7 0.1
9.3	25.9	1.5	17.3 0.1

Clone Design	Sequence
R20α-4-20C11-IR	XXXXXXXXXXXXXXXXXXXXX
R20α-3-20E2-IR	DRAFYNGLRDLVGAVYGAWD
R20α-4-20A12-IR	FYDAIDQLVRGSARAGGTRD
R20β-4-C6-IR	RLFYCGIQALGANLGYSGCV
R20β-4-A6-IR	FYSALWGLCGVTGCC
	RGQSDAFYSGLWALIGLSDG

FIG. 2C

Ratios over Background		Comparisons	
E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
--	--	--	--
39.0	19.2	--	--
33.4	17.5	--	--
31.8	14.3	--	--
37.8	13.5	--	--
37.2	10.0	--	--
37.0	9.5	--	--
34.2	9.4	--	--
30.3	7.8	--	--
20.8	6.9	--	--
32.8	6.5	--	--
35.4	6.4	--	--
25.0	6.4	--	--
23.4	6.3	--	--
31.3	4.0	--	--
13.6	3.3	--	--
16.8	3.2	--	--

Clone Design	Sequence
R20-4-F11-IGFR	GFYELLGALVGERVRCGTNS
R20-4-C7-IGFR	ERTDPFYKALLSLGGDGS
R20-3-F2-IGFR	DVQNGSSGFYDGI FGLAWG
R20-4-A11-IGFR	PFYVWIRDLLGPELPHTRGD
R20-4-B12-IGFR	VLVGGPLDPFYEGHLRLIS
R20-4-B10-IGFR	GFYRLNELVREGGALKVGA
R20-4-E9-IGFR	GQGFYELLSELLGHEGGVF
R20-3-H4-IGFR	DWVSGPFYRGIELLSGFQIE
R20-3-G2-IGFR	GGSLFYEGILRLVLGDSVVC
R20-4-B8-IGFR	LNHFYAMLSDLGVRNIFPG
R20-4-E7-IGFR	LSGFYEGFLRLARRDGSWG
R20-4-G9-IGFR	FYDVL SALVGVELGEQGDAS
R20-4-D9-IGFR	GAGSFGREGGFYEALMQLAG
R20-4-D11-IGFR	DDEFYSQILKLVDGSRGGRSGTON
R20-4-G10-IGFR	PFYMLLSRLVGGVEQEGGL
R20-4-C8-IGFR	FYDAIDQLVRGSARAGGTRD

FIG. 2D

Ratios over Background			Comparisons	
E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
---	---	---	---	---
31.9	1.4	2.1	0.7	1.5

Sequence

XXXXXXXXXXXXXXXXXXXXXXXXXXXX
RYFFPGGFYGNLDVLRWLRLPYVASPRWGHWRRPGGSLGKQPT

**Clone
Design
R40-3-40H4-IR**

FIG. 2A

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[illegible]

Sequence

AETPAQVGWNRLNSVWPGEHNTVPFYHKLSELLRESGA
 RHLTNAELGVQSPVLSRLFPDGDIFYRALSHLVRMGPP
 RGGMDRQWLVDVGARHLERRSVQDNTDDFYGGLRIILVDGF
 GPPDSFDVTEKGDMAILNVRFDPHSLDFNDQTFFYLLDSL
 GGTFRGQVAQSNESLLRVNLFQLLEALAAPPT
 APFDARLSAPRFQWSPRTWXQSLSYGEWSCGSFYDCLSSI
 MGSSQFQDTRPSSGQAYSHSLSDGCGGTANWIFLRALEGL
 SGAAHEGNQGRERSTHLAANINDHLPGDAGIWLGYSWLS

Clone	Design
RR40-3-B6-IGFR	
RR40-X-E5-IGFR	
RR40-X-B5-IGFR	
RR40-4-9-IGFR	
RR40-3-G6-IGFR	
RR40-4-12-IGFR	
RR40-3-A5-IGFR	
RR40-X-C6-IGFR	

FIG. 2B

Clone Design	Sequence X_n -Fy x WE- X_n	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
20E2B α -4-H5-IR	RSNDDAFYRWFSNIIQVDGGG	38.7	35.1	32.3	1.1	0.9	
20E2B α -4-G3-IR	DSDGAQFYIWFEDQLRSAGWD	35.5	36.1	32.7	1.1	0.9	
20E2B α -4-H4-IR	PGLHRAFYQWFAEAVRSANKE	38.8	37.9	35.0	1.1	0.9	
20E2B α -3-C1-IR	SLGQGGFYDWFASQVGGADI	43.7	42.1	39.0	1.1	0.9	34 / 200
20E2B α -4-E6-IR	CGQTQSFYQWFCVVRVESGD	38.0	34.3	29.7	1.2	0.9	
H5-3-D5-IR	IVVPGDTQGVNFYDWFVKQLQ	43.8	21.8	18.2	1.2	0.8	
JBA5-3-D9-IR	RDVSMGSASTNFYDWFVQQLG	38.3	29.8	25.3	1.2	0.8	
20E2B β -4-G6-IR	SQAGSAFYANFDQVLRVHSA	22.4	6.2	1.9	3.3	0.3	
20E2B β -4-H10-IR	SNGISGFYEWFAAQVQTSDFQ	23.5	32.2	9.7	3.3	0.3	
rB6-4-G8-IR	RRDRGGLDVFFYQWFMD	--	--	--	--	--	

FIG. 10-3

Clone Design	Sequence X_n -Fy x WF- X_m	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
20E2B α -3-D7-IR	TMGTQGFYRWFFQNVVKEHLSG	35.4	26.9	31.3	0.9	1.2	
20E2B α -3-A12-IR	ITHNRGFYSWFLDVVQGAGA	31.7	22.0	23.3	0.9	1.1	
20E2B α -3-D10-IR	VRRDAGFYQWFADILITQLDFE	32.7	27.3	29.1	0.9	1.1	
20E2B α -4-G7-IR	MQLQDEFYNWFRGIMLNDGQD	34.2	29.0	30.7	0.9	1.1	
20E2B α -4-F5-IR	GIRSSGFYQWFDRLVLAGVGDG	33.8	32.1	34.0	0.9	1.1	
20E2B α -3-C9-IR	ANLNSQFYSWFASVTGEASPS	39.4	33.2	35.5	0.9	1.1	
20E2B α -3-A4-IR	QSPRASFYGWFDLVLRAAGVV	38.2	31.6	35.9	0.9	1.1	
20E2B α -4-E12-IR	MQRNQGYSWFDDLVSSFTGV	36.0	30.8	29.7	1.0	1.0	
20E2B α -4-E11-IR	ASGFDPFYAWFLEQLRVANGS	35.1	31.2	30.7	1.0	1.0	
20E2B α -4-E8-IR	SGTPYGFYRWFFQSALASATSG	36.1	30.5	30.7	1.0	1.0	
20E2B α -4-H10-IR	QGVGEGFYEWFDRLMGDVRPW	38.9	30.6	30.7	1.0	1.0	
20E2B α -4-F6-IR	DNMSGGFYRWFFAQVVADSGGD	34.9	33.2	32.0	1.0	1.0	
20E2B α -4-G4-IR	RGTDDTFYGWFDQLLQGWCD	34.1	33.7	32.2	1.0	1.0	
20E2B α -4-F8-IR	TVDHTQFYDWFSLGESGSA	37.7	32.0	32.7	1.0	1.0	
20E2B α -4-G5-IR	GRQDRFYWFFELQAGGMDGD	34.9	33.9	33.4	1.0	1.0	
20E2B α -3-B10-IR	RLLGGFYEWFDQVLKETKEV	38.2	34.9	33.6	1.0	1.0	
20E2B α -3-C7-IR	GVLSTGFYEWFFALQLHGLAAG	37.6	34.2	34.8	1.0	1.0	
20E2B α -3-C5-IR	PAVGQSFYGWFFAVLRGSKAG	40.4	36.0	35.6	1.0	1.0	
20E2B α -3-B9-IR	SNGISGFYEWFFAAQVQTSDFQ	39.6	35.8	37.1	1.0	1.0	
A61-4-F11-IR	LLGLSQAAAYANFYDWFVSQLA	33.1	4.6	4.6	1.0	1.0	
20E2B α -3-C2-IR	VPNSWMFYWFFAEQIEGSEGE	44.1	40.0	38.1	1.0	1.0	
20E2B α -3-B2-IR	ARRADGFYDWFREQVSGSAVQ	43.1	40.1	39.0	1.0	1.0	
20E2B α -4-G2-IR	GVVEGTFYEWFDRLLGQVQGD	34.1	33.6	29.8	1.1	0.9	
20E2B α -4-H6-IR	SHLTDPFYQWFDVQDLRAGVRG	39.4	36.0	31.9	1.1	0.9	

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FIG. 10-2

CI n Design	Sequence $X_n - Fy_{WF} - X_m$	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
20E2A-3-B11-IR	GREYGFQDAIDQLMPWGEDP	24.6	1.4	23.6	0.1 16.8
20E2B-3-E3-IR	IQWEPFYGFDDVVAQMFEF	23.0	0.9	15.3	0.1 16.3
20E2B-3-F6-IR	RYGRWGLAQQFYDFWEDR	40.9	1.0	13.3	0.1 13.3
20E2B-4-F9-IR	RRLGSLSTQFYNWFAE	34.1	1.0	12.6	0.1 12.6
20E2B-3-A8-IR	ASATPFYQWFADVSEYMQQ	35.4	7.4	34.4	0.2 4.6
20E2B-4-F6-IR	PYRMETKWNFYDFWVAQLQ	28.9	4.1	18.1	0.2 4.4
20E2B-4-H9-IR	SAVHFQYKWFEDNLLFPPLSA	37.8	9.4	26.7	0.4 2.9
20E2B-3-B1-IR	VPVNSFYRWFLVLGGDDW	41.8	12.9	36.8	0.4 2.9
20E2B-4-F9-IR	QSPRASFYGFDDVLRAGV	25.9	4.2	10.1	0.4 2.4
20E2B-3-E9-IR	TGFYWFYEQHLSRMLPNPLD	27.0	7.7	17.2	0.5 2.2
20E2B-3-E10-IR	RRGVGGFYGFWSQQIQMGVA	22.2	2.6	5.5	0.5 2.1
20E2B-3-C12-IR	SSQDRRFYRWFEQAIVGGRDG	39.0	6.7	12.0	0.6 1.8
20E2B-3-C12-IR	TRGQLGFYWNFQALSTSGMG	20.2	2.2	3.8	0.6 1.8
20E2B-3-E7-IR	CADLNAFYQWFCGLDRGSDH	9.2	1.2	1.9	0.6 1.6
20E2B-3-E11-IR	TLIQDQFYWNFSDLLSAEPGD	20.7	1.3	2.1	0.6 1.6
20E2B-3-B11-IR	IDQLDAFYRWFDGVMIGMGDP	36.0	20.7	32.8	0.6 1.6
20E2B-3-B11-IR	RGGGTFYWFESALRKHGAG	10.8	6.3	8.9	0.7 1.4
20E2B-3-A7-IR	RGLDQDFYRWFNVLVGEYDR	19.0	4.2	5.5	0.8 1.3
20E2B-4-G12-IR	MQGHRGFYGFARVLEQDRGW	37.0	22.3	29.5	0.8 1.3
20E2B-3-C11-IR	ERLHLRFYWFDTVIGQDGS	37.3	26.8	34.8	0.8 1.3
20E2B-3-C10-IR	MHVQSDFYHWFQSILLQGGPD	37.7	24.8	30.5	0.8 1.2

FIG. 10-1

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	---
H2CB-4-H1-IGFR	xxxxxxfxxxfxxwxxxxxx	18.3	15.3	16.5	0.9	1.1	1.1
H2CB-4-F9-IGFR	HGVIRADHTGFYGFWSKQLSD	22.9	14.4	15.3	0.9	1.1	1.1
H2CB-4-E10-IGFR	LINA.VFRRGFYAWFEEQVSK	26.1	20.1	24.5	0.8	1.2	1.2
H2CB-4-F8-IGFR	LQRYIGFHPDFYDWFSAALSG	21.5	14.8	19.0	0.8	1.3	1.3
H2CB-3-A8-IGFR	MRTAELFHVGFDWFDALMD	20.7	14.7	18.2	0.8	1.2	1.2
H2CB-4-F1-IGFR	WAPPDALHGQFYRWFORQLDQ	22.2	14.6	18.8	0.8	1.3	1.3
H2CB-3-C6-IGFR	AVHAATFHDDFYRWFEQVVG	15.7	7.8	10.2	0.8	1.3	1.3
H2CB-4-E11-IGFR	FDAVHGFDDGFYGFWEKRELQR	26.1	17.6	24.1	0.7	1.4	1.4
H2CB-3-D6-IGFR	QAGMEFHGAFYNWFLQQLSG	21.6	13.0	18.8	0.7	1.5	1.5
H2CB-4-F3-IGFR	GRSVSRMNAEFYQWFGHQLAA	17.3	11.1	16.4	0.7	1.5	1.5
H2CB-3-A4-IGFR	AAVNSLFHDEFYLMFQDQLDG	27.4	11.0	14.8	0.7	1.3	1.3
H2CB-3-B1-IGFR	QLGMDWFHADFYEWFLAQLPS	20.0	11.0	15.2	0.7	1.4	1.4
H2CB-3-C5-IGFR	RLAGSGIHEGFYGFVFDQLLA	19.9	10.5	15.6	0.7	1.5	1.5
H2CB-4-F6-IGFR	GREIGGVHDGFYDWFRRQOSEQ	18.6	10.1	14.6	0.7	1.4	1.4
H2CB-3-B8-IGFR	VRSEQRFDSSFYQWFNDLIMS	20.7	6.9	9.5	0.7	1.4	1.4
H2CB-3-C7-IGFR	QSPYGFHHDGFYRWFLQQTGM	16.2	1.8	2.5	0.7	1.4	1.4
H2CB-4-H7-IGFR	FQCGAAFHVDFYRWFTCQEQF	21.8	14.1	22.7	0.6	1.6	1.6
H2CB-4-F5-IGFR	GAFGSEFHEQFYRWFEALSF	12.9	4.0	7.2	0.6	1.8	1.8
H2CB-4-G1-IGFR	EHTSYQIHRQFYEWFDALGR	20.4	10.3	19.7	0.5	1.9	1.9
H2CB-3-D11-IGFR	SGTAADLHSRFYGFWALQARE	24.1	8.8	18.6	0.5	2.1	2.1
H2CB-3-D7-IGFR	EGFGVLFHGQFYRWFLQLDQ	22.1	6.5	13.6	0.5	2.1	2.1
H2CB-3-C10-IGFR	QOSAGHPHSSFYLMFSEILGA	21.7	5.1	10.4	0.5	2.0	2.0
H2CB-4-E3-IGFR	YLQIRAGFHRSFYGFWDQALRD	20.3	4.6	8.9	0.5	1.9	1.9
H2CB-3-C1-IGFR	NMLWATLHSDFYSWFEQVVG	22.3	6.7	15.7	0.4	2.3	2.3
H2CB-4-G2-IGFR	GANALGFKDRFYEWFAAQLWD	19.9	3.3	10.7	0.3	3.3	3.3
H2CB-3-A11-IGFR	GSGLYVEHWGFYDWFEEQMG	23.9	2.5	7.7	0.3	3.1	3.1
H2CB-4-G5-IGFR	LDKGWGFDLQFYRWFEAATRA	19.3	2.5	7.9	0.3	3.1	3.1
H2CB-4-F12-IGFR	QRSAYEFHADFYDWFELRLTP	16.7	1.7	5.4	0.3	3.1	3.1
	DQRMGSFHFGEFYRWFEETLLS						

FIG. 1N-3

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
H2CB-4-H10-IGFR	XXXXXXXXXXXXXX	21.9	22.4	1.0	1.0
H2CB-4-H5-IGFR	GALSSLFDAAFYDWFNRQLEG	22.3	22.3	1.0	1.0
H2CB-4-G7-IGFR	KVDLRGFHDGFGYGFARQLAG	23.1	21.6	1.0	1.0
H2CB-4-F4-IGFR	CSGLQRCHDSFYSWFESVURE	21.3	20.9	1.0	1.0
H2CB-3-D8-IGFR	DSLGISFHGFGFYDWFRRQIDM	20.0	20.5	1.0	1.0
H2CB-4-E4-IGFR	SGVFNGTFYDWFRIQLGE	21.6	20.5	1.0	1.0
H2CB-4-E5-IGFR	GYREMRSDLGFGYQWFRDQLGL	22.0	19.9	1.0	1.0
H2CB-4-E8-IGFR	SVFMQHDHVGFGYAWFRSIMEE	21.1	19.7	1.0	1.1
H2CB-3-D12-IGFR	FRHITVEDRSFYGWFVEQLRG	26.6	17.3	1.0	1.0
H2CB-4-G9-IGFR	WAGGSDVDGSFYDMFQRLLAS	21.6	14.5	1.0	1.1
H2CB-3-C8-IGFR	GLQNVSEHSGFYEWFRQVSQ	20.8	13.4	1.0	1.0
H2CB-3-A12-IGFR	SRVSDPYHVFGFYQWFEVVRG	28.6	27.5	0.9	1.1
H2CB-3-B12-IGFR	MGGATFHTGFGYDWFQAQLQH	27.8	25.2	0.9	1.1
H2CB-3-A9-IGFR	RPASRPFHSGFYQWFAADQLSH	27.7	24.3	0.9	1.1
H2CB-3-A3-IGFR	GLAPGNFHEDFYRWFEQTLG	26.9	24.1	0.9	1.1
H2CB-3-B4-IGFR	TAAISDFNSLFYGFWEQLLSS	25.8	23.8	0.9	1.1
H2CB-4-E7-IGFR	LDDELPOHAGFGYGFWEALGV	24.6	21.6	0.9	1.1
H2CB-4-G6-IGFR	ASHKSAFDNDFYRWFSMQLRD	22.4	21.1	0.9	1.1
H2CB-4-E9-IGFR	HTGAGDLHGAFYNWFLEQLGG	24.3	20.7	0.9	1.1
H2CB-4-H2-IGFR	RRGRDGFHGGFYDWFQAQLSD	21.6	20.2	0.9	1.1
H2CB-3-A10-IGFR	GNFREATHADFYSWFEQLQS	24.3	19.9	0.9	1.1
H2CB-3-C4-IGFR	RDILPAFHQHFYQWFEKQVSA	23.1	19.2	0.9	1.1
H2CB-3-B5-IGFR	ERETAAGQAFYQWFRDQIAG	24.2	18.8	0.9	1.1
H2CB-4-G4-IGFR	WGEGGGFYDWFYDQLGWEP SH	21.7	18.7	0.9	1.1
H2CB-3-D9-IGFR	SLVAADLHEGFGYGFWSQLGG	24.4	18.6	0.9	1.1
H2CB-3-C3-IGFR	TSEVGDFHAEFYSWFEIQLGR	20.3	18.4	0.9	1.1
H2CB-3-D3-IGFR	TCADGLLHARFYAWFEELIRE	22.5	18.3	0.9	1.2
H2CB-4-F2-IGFR	RRSDSSLHRSFYDWFVSQLLN	18.0	16.8	0.9	1.1
	SESKYLLHSCFGYGFWEAQLRG				

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental	XXXXXXXXXXXXXX	29.8	17.5	16.3	1.1	0.9
H2CB-3-D2-IGFR	VTFTSAVFHENFYDFWFRQVS	22.9	18.6	11.8	1.6	0.6
H2CB-3-C12-IGFR	TASQECFFDDGYGFWFAWRCT	17.3	19.6	13.0	1.5	0.7
H2CB-3-B11-IGFR	SLDWRWSEEPFYRFQALAG	24.6	17.1	11.9	1.4	0.7
H2CB-4-E2-IGFR	CMSLSDCHRKFGYGFKSQGE	22.4	21.0	16.5	1.3	0.8
H2CB-3-A5-IGFR	LALCRRSPGSFYGFQAAGVC	28.8	26.1	22.6	1.2	0.9
H2CB-4-G12-IGFR	PRSATMSDGGFYWWFASQLGL	23.7	23.8	19.4	1.2	0.8
H2CB-3-B2-IGFR	LRRSSVFHDPFYE*ISRLVGG	23.0	19.9	16.4	1.2	0.8
H2CB-3-D1-IGFR	ARLQQQFHGGFYENFRAQVSP	21.5	19.5	15.7	1.2	0.8
H2CB-3-B6-IGFR	AQLDNLCHEPFYSWFCVATRE	16.3	4.5	3.7	1.2	0.8
H2CB-4-F7-IGFR	WTCDTAFHQDFYQWFCDKLGV	22.0	19.0	18.0	1.1	0.9
H2CB-4-G8-IGFR	GKEGFLDRDFYWWFREQLGP	20.2	18.6	16.5	1.1	0.9
H2CB-3-D4-IGFR	GRAPSSFDGDFYCWFRNQVS	21.9	18.3	16.9	1.1	0.9
H2CB-3-D5-IGFR	DVEAETQHLFYAWFLSQLGS	21.4	17.9	16.4	1.1	0.9
H2CB-4-E6-IGFR	ISVTAVFHDGFGYGFNEQVSK	19.6	15.8	14.8	1.1	0.9
H2CB-3-C2-IGFR	NSEHGRLDVDFYGFWARVIOQ	18.8	12.2	10.8	1.1	0.9
H2CB-3-A6-IGFR	GPLGDCQDGFYGFWMFCQVST	26.8	29.0	28.1	1.0	1.0
H2CB-4-H12-IGFR	KRSAYNFHDPFYDWMFRMLSG	23.9	28.3	28.1	1.0	1.0
H2CB-3-B10-IGFR	ASEPGGYLDPFYGFWRFLQRA	27.1	27.5	27.3	1.0	1.0
H2CB-4-F11-IGFR	NRDGGVHSGFYWNFRLLQSG	25.5	25.5	24.6	1.0	1.0
H2CB-4-G11-IGFR	ASKGSSLHNDFYGFWFAOQLAR	25.3	25.4	25.3	1.0	1.0
H2CB-4-E12-IGFR	ANVSMWIOVGFYDWFDAQLRQ	27.8	24.9	24.7	1.0	1.0
H2CB-4-G10-IGFR	RTSPGSLHDPFYDWFQQQLGG	25.1	24.6	24.2	1.0	1.0
H2CB-3-B9-IGFR	PGVMSFHHGGFYSWFREQLNG	25.6	23.3	23.7	1.0	1.0
H2CB-3-B7-IGFR	CLANSEHDHSFYGFWFCQALGG	24.0	23.2	23.5	1.0	1.0
H2CB-4-H4-IGFR	GGSMGGMHGSFYEWFAQLRS	23.5	23.1	23.8	1.0	1.0
	RPQGGSIHAGFYQWFRDAVAG					

FIG. 1N-1

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGFsR --	IGFR/IR --	IR/IGFR --
H2CBβ-3-E8-IR	TGHR1GLDEQFYWWFRDALSG	15.9	1.9	11.8	0.2
H2CBβ-4-F8-IR	VLTSNTLHQRFYSWFAAARRE	13.4	0.8	2.6	0.3
H2CBβ-3-C4-IR	CVAQGGFQSSFYCWFAGLDID	21.1	1.3	4.0	0.3
H2CBβ-3-D5-IR	NGOSSRFHTAFYDWF FAAQLSG	14.0	3.3	10.2	0.3
H2CBβ-3-E6-IR	SVPRGTVHDAFYQWFREVALG	5.7	0.7	2.1	0.3
H2CBβ-4-G12-IR	GARGSTFHDQFYEWFWVQLGD	6.8	1.8	5.4	0.3
H2CBβ-4-F4-IR	PPGMNGFHTSFYSWFDVQLGD	17.9	1.9	5.6	0.3
H2CBβ-4-F11-IR	AVGTLGYHSGFYRWFERQLGG	15.0	1.7	4.8	0.3
H2CBβ-3-E5-IR	ELQARGVHRNFYRWFEAQVSG	17.0	1.8	5.0	0.4
H2CBβ-4-F2-IR	HRVARAFHEQFYDWF FEKAVSG	15.9	1.3	3.4	0.4
H2CBβ-4-G4-IR	GAMEPDYHRSFYQWFAAALGE	8.7	1.4	3.5	0.4
H2CBβ-3-C8-IR	CPDRQSVDDRFFYNWFADALAS	4.9	1.4	3.2	0.4
H2CBβ-4-F10-IR	GGAQISFHERFYQWFLQEAAG	10.2	1.0	2.4	0.4
H2CBβ-4-H4-IR	HKRGIVQHGAFAWFD SLLSG	20.8	4.2	9.5	0.4
H2CBβ-4-G6-IR	QASDNRSDGQFYLWFEKLSS	14.5	5.6	8.5	0.7
H2CBβ-4-H1-IR	DRGRMGVDEGFYNWFARQME	17.0	10.1	13.2	0.8

FIG. 1M-5

Cl ne Design	Sequence	Ratios over Background			Comparisons	
		E-Tag --	IGFsR --	IR --	IGFR/IR --	IR/IGFR --
H2CBα-3-D6-IR	XXXXXXXXXXFYXWFXXXXXXXX	27.9	26.0	25.8	1.0	1.0
H2CBα-3-H3-IR	AGVMGGFHQEFYLWFERALSN	27.0	26.9	26.2	1.0	1.0
H2CBα-3-F4-IR	AGHVQVYDGYGWFREQLGA	31.2	27.2	27.7	1.0	1.0
H2CBα-3-E9-IR	FVQNI GFDYDFYGFVREVEK	31.6	27.7	28.2	1.0	1.0
H2CBα-3-H10-IR	PVGIGGLHRAFYQWFQSQVDA	26.9	27.9	28.8	1.0	1.0
H2CBα-3-G2-IR	GSRQEADHQAFYDWFNLVLGV	29.1	28.1	28.8	1.0	1.0
H2CBα-3-B2-IR	AGGRKPFHDDFYGWFRRDQLAE	29.4	28.1	28.2	1.0	1.0
H2CBα-3-E8-IR	DLASHGFHDAFYNWFVSQVINS	31.5	28.4	29.1	1.0	1.0
H2CBα-3-E5-IR	GSNGGGVHGQFYAWFVEALSG	33.0	28.7	28.9	1.0	1.0
H2CBα-3-E6-IR	RGRASTFHDGFGYGFWSQQLRF	29.6	29.0	28.1	1.0	1.0
H2CBα-3-E7-IR	SSDVGAHSAFYDWFKAQLSG	30.4	30.2	30.2	1.0	1.0
H2CBα-3-C8-IR	PTVHRAFDDDLFGWFQAKQVED	31.9	31.2	31.5	1.0	1.0
H2CBα-3-A4-IR	SSNTVGLDERFYAWFVDQLGA	32.2	31.9	32.6	1.0	1.0
H2CBα-3-D1-IR	PGAAEGFHSAFYDWFAQAVSG	32.9	32.5	31.5	1.0	1.0
H2CBα-3-B9-IR	MRSEASFHVEFYSWFEEQLRS	33.2	33.8	33.3	1.0	1.0
H2CBα-3-D8-IR	VSRYGGQQDGFYHWFSDLLKG	26.3	20.2	19.1	1.1	0.9
H2CBα-3-F1-IR	RPSSGGLHYGFYHWFVRVQEEM	28.8	28.0	26.4	1.1	0.9
H2CBα-3-A11-IR	SNTEEHFHMQFYRWFSDALGN	20.5	21.5	17.7	1.2	0.8
H2CBα-3-A3-IR	ANDCLGLHAGFYGNFACOLGG	30.4	29.6	21.8	1.4	0.7

FIG. 1M-4

Cl ne Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
H2CB α -3-A9-IR	XXXXXXXXXXXXXXXXXXXXX	30.5	21.7	24.1	0.9	1.1
H2CB α -3-C11-IR	RVDAAALNAGFYEWFRGVIQG	26.4	21.8	23.2	0.9	1.1
H2CB α -3-B4-IR	GGAGRSFHDFAFYEFERQMAG	30.9	22.0	24.3	0.9	1.1
H2CB α -3-F11-IR	EGARQGFHARFYSWFAQQLAL	24.5	22.5	23.9	0.9	1.1
H2CB α -3-G10-IR	VLLPGVVHGGFYDWFQRQLSS	28.3	23.6	27.1	0.9	1.1
H2CB α -3-D7-IR	GALSDRYNNVFYDWFREQLLG	31.4	23.6	25.3	0.9	1.1
H2CB α -3-E2-IR	PDSFMSLHQRFYSWFQAQVGT	26.8	24.0	25.7	0.9	1.1
H2CB α -3-B5-IR	RVYKANFHNEFYGFWRFEQLLG	28.7	25.0	26.4	0.9	1.1
H2CB α -3-C7-IR	HSGMRDVHARFYSWFSEQLSG	30.0	25.2	28.7	0.9	1.1
H2CB α -3-G9-IR	ARLLERFQDPFFYEFWFTLMGD	27.8	25.2	26.7	0.9	1.1
H2CB α -3-A12-IR	RNSSGNFHDKFYNWFEAQLKG	28.0	26.4	28.7	0.9	1.1
H2CB α -3-C9-IR	GSMSPVENDQFYGWFRDLVDE	32.1	28.7	31.9	0.9	1.1
H2CB α -3-B10-IR	SCTGRQFDGCFYAWFEDQLVG	33.5	30.8	33.2	0.9	1.1
H2CB α -3-E1-IR	GIAVQSLHDSFYRWFEDNALGS	31.7	30.5	29.0	1.1	1.0
H2CB α -3-G12-IR	IGPPGSLHRGFYDWFQAQVEA	29.1	31.4	29.8	1.1	1.0
H2CB α -3-F7-IR	GAAGISFHRGFYDWFQAQVRD	23.2	20.7	20.3	1.0	1.0
H2CB α -3-G8-IR	GVDVTDHFKDFYSWFQRQLNG	22.8	20.9	20.4	1.0	1.0
H2CB α -3-C6-IR	WAGRAGIHGGFYEFWFRQLRG	26.7	21.2	22.0	1.0	1.0
H2CB α -3-H9-IR	LGQLAAFHGLGFYEFWFEAVAA	23.4	22.5	22.0	1.0	1.0
H2CB α -3-H8-IR	VHSVSRNLNVGFYQWFQDQLSG	23.5	23.4	23.2	1.0	1.0
H2CB α -3-F2-IR	IGLMAIFDRGFYGFWEQQLSG	25.5	24.3	25.2	1.0	1.0
H2CB α -3-D5-IR	VARGSSLHDDFYEFWFEASQLRT	26.7	24.5	25.6	1.0	1.0
H2CB α -3-D10-IR	LGYGALNTQFYSWFADLVGS	26.8	24.9	24.9	1.0	1.0
H2CB α -3-F10-IR	EDSRLRLHEGFYGFWRKQLGD	25.7	25.6	26.1	1.0	1.0
	GRDNMKFHSGFYDWFQTQLAG					

FIG. 1M-3

Cl ne Design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGFR --	IR --	IGFR/IR --
H2CBα-3-D11-IR	XXXXXXFHHXXFYXWFXXXXXX	27.8	13.0	24.8	0.5
H2CBα-3-H12-IR	GPGRQLHDAFYSWFDALRVN	27.4	7.2	12.4	0.6
H2CBα-3-A10-IR	LGTAVFHELFGWFERQLGG	27.1	13.2	22.3	0.6
H2CBα-3-A5-IR	GGYCGFNCQFYRWFNDLADR	28.3	16.1	28.1	0.6
H2CBα-3-C4-IR	FSGWADYQSGFYQWFAEELAN	30.7	17.2	29.2	0.6
H2CBα-3-B8-IR	WGPFVSFDESFYRWFQAASDD	25.6	11.3	18.6	0.6
H2CBα-3-H11-IR	PRNEGLVHGLFYDWFORALSG	28.8	14.0	22.4	0.6
H2CBα-3-E10-IR	DEGGAPLDVMFYRWFQAVRG	27.7	14.3	23.0	0.6
H2CBα-3-C2-IR	QSGNRGSHGAFYSWFRDVLAN	28.4	17.0	26.7	0.6
H2CBα-3-F6-IR	MRQRDGFNSSFYGWFAAALGE	27.3	14.5	21.8	0.7
H2CBα-3-D4-IR	SEERKKVHSQFYSWFDRQLLG	29.0	18.9	27.1	0.7
H2CBα-3-A7-IR	PSPNAPFHGGFYDWFDWVQGS	29.1	19.4	26.9	0.7
H2CBα-3-H4-IR	FHRPGSFNTNFYQWFDQMNQ	27.2	20.1	27.9	0.7
H2CBα-3-B7-IR	SDDSTLNGRFYTWFMQLLD	28.6	18.0	23.6	0.8
H2CBα-3-F9-IR	QRGGGFHEGFYSWFRSQSLL	26.1	19.1	24.3	0.8
H2CBα-3-H6-IR	SGSRPVTHEQFYEWFDQLGL	24.8	21.6	27.3	0.8
H2CBα-3-F5-IR	GGSSQAFHGAFYEWFSQLRG	29.4	22.0	27.8	0.8
H2CBα-3-A2-IR	AFVSENVNQRFYDWFRDQMR	30.7	22.5	29.1	0.8
H2CBα-3-F3-IR	VRHPTRFHDEFYRWFTQLTT	16.3	6.7	9.0	0.7
H2CBα-3-G6-IR	ARLLNIFDRGFYNWFQRQLDE	24.9	21.0	24.4	0.9
H2CBα-3-G7-IR	PSLSSNLHESFYRWFDQLVST	24.4	18.7	23.0	0.8
H2CBα-3-C5-IR	FAPGLGFHQFYDWFAHQLEG	26.4	21.2	25.4	0.8
H2CBα-3-G1-IR	VSATVNLHREFYDWFGQLLD	26.9	21.5	26.3	0.8
H2CBα-3-E3-IR	GGVSGVLHDFYSWFERQLAG	24.2	17.2	19.3	0.9
	GLGLASFHEGFYSWFTAQLGA				1.1

FIG. 1M-2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
Parental	XXXXXXXXXXXXXXXXXXXX	--	--	--	--
H2CB α -3-B12-IR	VTFTSAVFHFNFYDFWFRQVS	29.8	17.5	1.1	0.9
H2CB α -3-D2-IR	QSDSGTVHDFYGFWRDT*A	26.0	1.3	0.1	16.0
H2CB α -3-D12-IR	WTDVDGFHSGFYRWFRQWER	20.6	1.7	0.1	7.0
H2CB α -3-H5-IR	VASGHVLHGQFYRWFRVDQFAL	24.6	2.1	0.1	6.7
H2CB α -3-B6-IR	QARVGNVHQFYWFREVMQG	16.7	2.4	0.2	6.3
H2CB α -3-G11-IR	VGDFCVSHDCFYGFWRFLRESMQ	31.4	2.5	0.2	5.6
H2CB α -3-A6-IR	SGSRPVFHEQFYWFVDQLG	22.7	1.4	0.2	4.7
H2CB α -3-B1-IR	QFSAGAFHGDYGFWRFRALYNG	25.9	1.7	0.2	4.3
H2CB α -3-F8-IR	SRFDERLHHQFYWFVRVLNRP	33.4	6.0	0.2	4.3
H2CB α -3-E11-IR	DSVNSDLHRAFYGWFAEQWRA	23.0	4.8	0.2	4.1
H2CB α -3-G4-IR	GSVDREIHGPFYSWFSEQLWG	14.0	2.2	0.3	4.0
H2CB α -3-D3-IR	SAKTPVLHDGFYWFEEAQSES	24.9	2.2	0.3	3.2
H2CB α -3-C1-IR	LVVGRRFHQSYDFWFAAAGG	23.6	2.6	0.3	3.1
H2CB α -3-C3-IR	IMWPCTFQDPPFYCWFTQEQGR	27.0	5.6	0.3	2.9
H2CB α -3-G3-IR	VVGPLDIHERFYGWTHQQGGA	23.3	1.1	0.4	2.8
H2CB α -3-E4-IR	VVPKAGFHEAFYWFRRQRDRD	23.7	6.7	0.4	2.6
H2CB α -3-G5-IR	QSEFVTSVHTRFYAWFASALEM	28.8	8.3	0.4	2.6
H2CB α -3-B11-IR	SRGLGLYHSGFYGWFERQFNQ	26.7	7.0	0.4	2.5
H2CB α -3-A1-IR	GADTGAVHRRFYLWFEQLSGG	28.0	8.6	0.4	2.3
H2CB α -3-H1-IR	PGNRPTFHAFFYRWFRFAQGS	31.3	11.3	0.5	2.2
H2CB α -3-F12-IR	VAVANGLHESFYAWFENQFSD	27.2	10.6	0.4	2.2
H2CB α -3-H7-IR	GFNTGTFFHDQFYWFWEAAGG	21.1	6.1	0.5	2.1
H2CB α -3-C12-IR	GDGLTAFHQGFYWFEDIQMYG	21.0	9.7	0.5	2.0
	VGVNRQFHTRFYAWFEDEQLGG	26.0	12.7	0.5	1.9

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FIG. 1M-1

Cl ne Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
H2CA-3-E6-IGFR	XXXXXXXXHFNFDWDFVROVSXXXXXX	--	--	--	--
H2CA-4-F4-IGFR	WRHGTFHEDEYDWFVROVSGSGSST	15.7	8.7	3.1	2.8
H2CA-3-D10-IGFR	GGRVGVLFHENFYDWFDRQVSLRGADG	11.5	7.4	3.0	2.5
H2CA-3-E1-IGFR	CNLTAGFHEQFYHWFVFAIQVCGDAENA	9.4	6.8	2.9	2.3
H2CA-2-B6-IGFR	ERGEDMFHENFYDWFVROISGRQGGG	12.5	6.4	2.8	2.3
H2CA-3-E11-IGFR	TNQGVGFYDSFYGWFVROIQYGVDSG	18.0	6.2	2.7	2.3
H2CA-4-H2-IGFR	HLADGQFHEKFYDWFERQISSRCNDC	4.7	2.2	1.0	2.2
H2CA-3-C11-IGFR	QTFGKSLHENFYDWFVROVSREEGGD	9.8	9.9	4.8	2.1
H2CA-2-B8-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3.3	1.6	2.1
	SASTHQFHENFYDWFVROVSGAQKIL	14.6	7.9	3.9	2.0

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FIG. 1L-2

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Cl ne Design Parental	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFR	IGFR/IR	IR/IGFR
H2CA-4-G9-IGFR	XXXXXXFHENFYDFVFRQVXXXXXX	29.8	17.5	16.3	1.1
H2CA-4-H6-IGFR	VTFTSAVFHENFYDFVFRQV	8.6	9.5	0.6	16.0
H2CA-4-F-IGFR5	GLISQSCPESEFYDFWFGQVSDPWWCW	4.9	10.5	0.7	14.6
H2CA-4-H8-IGFR	VGRASGFPENFYDFWFGRLSLQSGEQ	5.5	9.7	0.8	12.3
H2CA-4-F11-IGFR	VGYQGQDENFYDFWFIQVSGRLGVQ	5.6	9.2	1.0	9.4
H2CA-4-F6-IGFR	SACQFDCHEFYDFWFIQVSGGAAYG	3.5	6.8	1.0	6.7
H2CA-4-F10-IGFR	SAAQLFFQESFYDFWFLRQVAESSQPN	3.9	7.3	1.1	6.4
H2CA-1-A3-IGFR	AVRATRFDEAFYDFWFRQISDQGNK	4.9	5.7	1.0	5.9
H2CA-3-C8-IGFR	VNQSGSIHENFYDFWFERQVSHQGV	7.7	3.8	0.8	5.1
H2CA-2-B9-IGFR	APDPSDFQEIFYDFWFRQVSRMPGGG	15.1	5.6	1.2	4.8
H2CA-4-H4-IGFR	SSCDGAGHSEFYDFWFRQVSGCRSV	9.3	7.0	1.7	4.2
H2CA-4-F7-IGFR	RAGSSDFHEDFYDFWFRQVSLSLKKG	3.9	4.1	1.0	4.2
H2CA-3-D6-IGFR	QAVQPGHEEFYDFWFRQVSTGVGGG	1.5	3.2	0.8	4.1
H2CA-4-G11-IGFR	SSIGGTFHENFYDFWFSRQLSQSPPIK	8.3	9.0	2.2	4.0
H2CA-4-F1-IGFR	QSPVGSSEHDFYDFWFFRQVAGSAHQ	10.9	7.2	1.8	4.0
H2CA-3-D7-IGFR	NYRRQVFNENFYDFWFRQVSLVTPG	10.8	9.5	2.5	3.9
H2CA-1-A7-IGFR	TLDGGSFEEQFYDFWFRQVSYRTNPD	5.8	3.5	0.9	3.8
H2CA-2-B3-IGFR	FXVQOQGHENFYDFWFRQVSGGAG	13.3	3.0	0.8	3.7
H2CA-2-B2-IGFR	LRRQAPVEENFYDFWFRQVSGDRVGG	8.0	2.2	0.6	3.7
H2CA-3-D4-IGFR	RCGRLYHSTFYDFWFRQVAGRTCP	3.5	4.1	1.1	3.6
H2CA-4-F2-IGFR	CCLLCRFQNFYDFWFRQVSGRGG	7.7	3.8	1.0	3.6
H2CA-3-D11-IGFR	PPLASDLVDQFYDFWFRQVQAATG	4.1	3.4	1.0	3.5
H2CA-4-H9-IGFR	RSASGSLPEQFYDFWFRQVSLSGTDK	17.6	13.8	4.1	3.4
H2CA-2-B11-IGFR	SRVTVFHENFYDFWFRQVSLSDAISG	9.3	12.8	4.2	3.0
H2CA-3-E8-IGFR	DERGGKREDFYDFWFRQVSESRFGQ	12.2	6.9	2.3	3.0
	RGAVAGFHDQFYDFWFRQVSRVHKFG	8.7	5.6	1.9	3.0
	AICDAGFHEHFYDFWFRQVSDCGRQS	11.9	4.6	1.6	3.0
	LGYPEPQQNFYDFWFRQVSGAENAG	13.2	6.3	2.2	2.9

FIG. 1L-1

Ratios over Background		Comparisons	
E-Tag	IGFsR	IGFR/IR	IR/IGFR
--	--	--	--
35.9	34.7	1.5	0.7
38.7	37.6	1.3	0.7
37.8	19.6	2.0	0.5

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Sequence
 XXXXXXFFHENFYDWFVRQVSXXXXXX
 RAGGVGLHDNFYDWFVRQVSGGDSGP
 ADCYVQLHENFYDWFVRQVCNLQEGM
 RQCHAGFHDNFYDWFVRQVSGSTPQV

Clone
 Design
 H2CA-4-G7-IR
 H2CA-3-C6-IR
 H2CA-3-B8-IR

FIG. 1K-4

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Cl ne Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
H2CA-3-D12-IR	SEVDSDSRHENFYDWFVRQVSGIGLQD	36.8	34.1	1.2	0.9
H2CA-3-B5-IR	PAPADAFDHNFYDWFARQLSATTIQ	38.8	35.2	1.2	0.9
H2CA-4-E1-IR	MVQRISEHENFYDWFVRQISGSAVPP	29.8	12.5	1.1	0.9
H2CA-3-D3-IR	GNVRGQFHGFYDWFARQVSGSEGDA	33.1	29.9	1.1	0.9
H2CA-4-E3-IR	PDAEKQFHETFYDWFVRQISEDSANS	33.3	32.3	1.1	0.9
H2CA-4-E12-IR	FGRGVHCDEFYDWFVCQVSGALLEG	36.0	32.4	1.1	0.9
H2CA-3-A6-IR	ETPLTELHEQFYDWFVRQVSGFFPGV	34.0	33.1	1.1	0.9
H2CA-4-E9-IR	QHRGPHFHEDEFYDWFVRQVSSAVPSD	38.8	33.7	1.1	0.9
H2CA-4-F3-IR	RQDPGLFHDNFYDWFDRLVSAWDQE	41.0	34.2	1.1	0.9
H2CA-4-H6-IR	QAAVGVCNKDFYAWFACQVREDEFAK	37.1	34.5	1.1	0.9
H2CA-4-H2-IR	RNNLQFNENFYDWFDRQVSAIRGGG	41.8	35.3	1.1	0.9
H2CA-3-D4-IR	RSEQYRFHENFYDWFDRQVSRMGLLG	38.7	35.5	1.1	0.9
H2CA-3-D1-IR	GAGGRDFDEDFYDWFVRQVSGQVTS	34.5	35.5	1.1	0.9
H2CA-3-C1-IR	SPEGNLVHDQFYDWFVRQLSSTSAGT	39.9	36.1	1.1	0.9
H2CA-3-D8-IR	QGGGLGDFDEDFYDWFARQVSRDRAD	37.8	36.7	1.1	0.9
H2CA-4-H4-IR	LSQGVGFQENFYDWFVRQVSGWDGRD	38.5	37.0	1.1	0.9
H2CA-4-F6-IR	VFERSRCHDNFYDWFVCQVSGQADGG	38.7	37.5	1.1	0.9
H2CA-4-E4-IR	LLASRAFHENFYDWFARQVSGTQPPG	38.6	38.0	1.1	0.9
H2CA-3-C11-IR	VPDAQIFHESFYDWFVRQASAGGPAD	40.3	38.3	1.1	0.9
H2CA-3-C4-IR	ANQMRFDHNFYDWFDRQVSRYERGT	41.9	38.4	1.1	0.9
H2CA-4-E6-IR	PSRKDGLHQSFYDWFARQVQDMEGRA	39.3	38.8	1.1	0.9
H2CA-3-D7-IR	QAVTRRFHENFYDWFARQVSEEGGWS	42.5	39.2	1.1	0.9
H2CA-3-A7-IR	GYAVGQYQANFYDWFVRQVDGMSNGG	35.3	15.2	1.3	0.8
H2CA-4-G12-IR	GHQRDLLHESFYDWFVRQVSEAEAGG	37.6	19.4	1.3	0.8
H2CA-3-D6-IR	DRPSSFIHENFYDWFARQVSGSSSG	39.4	36.2	1.3	0.8
H2CA-4-H12-IR	ERTAEHLHEQFYDWFVRQVSAWDGES	40.0	38.4	1.3	0.8
H2CA-3-D11-IR	LTSQLLSHEDFYDWFVRQVSGVGGSG	38.1	32.9	1.2	0.8
H2CA-3-C12-IR	PDRSDRLDDNFYDWFVRQVSGVINED	38.5	38.4	1.2	0.8

FIG. 1K-3

Clon Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
H2CA-3-D9-IR	XXXXXXXXXXXXXXXXXXXXX	36.6	30.6	30.9	1.0 1.0
H2CA-3-C2-IR	VSGRGAFHENFYDWFVRQVFRDEQDT	38.3	30.7	31.0	1.0 1.0
H2CA-4-G1-IR	ARPPPTVHENFYDWFVRQVSETWRQD	34.1	30.7	30.4	1.0 1.0
H2CA-4-E2-IR	QGGDRLFHERFYDWFDRVLSSDSTGE	33.9	31.0	31.8	1.0 1.0
H2CA-4-H9-IR	QHIAAGLHENFYDWFIRQVSGVNPVA	38.9	31.1	31.4	1.0 1.0
H2CA-3-D2-IR	QPNDDLHENFYDWFVRQVSNVAVDGG	33.0	31.1	29.8	1.0 1.0
H2CA-3-B3-IR	PVEFTVYHDNFYDWFARQVSDGLGQF	35.3	31.4	30.0	1.0 1.0
H2CA-4-G11-IR	FCVQASIHENFYDWFVRQVAENQVFS	37.9	31.9	31.0	1.0 1.0
H2CA-4-F2-IR	GRPRGSFHENFYDWFARQVSGDGAGT	38.1	32.0	31.9	1.0 1.0
H2CA-3-C5-IR	IVGASLCHESFYDWFACQVTLNLSQGG	36.9	32.3	31.6	1.0 1.0
H2CA-3-B2-IR	IGLRQMFHENFYDWFAREVSKEAGDG	36.6	32.7	32.5	1.0 1.0
H2CA-3-B11-IR	LGGAIEGHGNFYDWFVRQVSLDVGGE	35.5	32.8	33.3	1.0 1.0
H2CA-4-G2-IR	LNALQQLHENFYDWFEGRQVSAFPPGG	35.9	33.0	33.4	1.0 1.0
H2CA-3-A4-IR	VGNCDTFPENFYDWFACQVSELGGMN	33.3	33.0	32.9	1.0 1.0
H2CA-4-H3-IR	FSQDGNFHENFYDWFDRQLSLVGAGT	39.5	33.7	33.7	1.0 1.0
H2CA-4-G5-IR	PAGNRALHESFYDWFVRQVSEFQLGA	35.3	34.0	35.6	1.0 1.0
H2CA-4-E8-IR	DLRLARFNFENFYDWFDRQVSGQSGMP	35.7	34.7	34.9	1.0 1.0
H2CA-4-G6-IR	VLGVAQFHDKFYDWFARQVSLQESAG	36.2	35.0	33.5	1.0 1.0
H2CA-3-B7-IR	GVVGGAFHEQFYDWFDRQVSAAFKGD	37.6	36.5	35.3	1.0 1.0
H2CA-3-B4-IR	DESEMRLHEQFYDWFARLVSLGEGSA	39.8	36.5	35.1	1.0 1.0
H2CA-3-C7-IR	EGGGVAIHENFYDWFDRQVSLQGNWD	40.2	36.7	35.9	1.0 1.0
H2CA-4-E5-IR	SRIVSREHENFYDWFVRQVSGDAPVQ	37.3	37.0	36.3	1.0 1.0
H2CA-4-E7-IR	IPAGAQLHENFYDWFARQVSGEDGGA	39.7	37.6	37.6	1.0 1.0
H2CA-3-B9-IR	GSSAAGFDEQFYDWFDRQVSEAFRDG	39.4	37.7	37.6	1.0 1.0
H2CA-4-F5-IR	RLAIRTFHQDFYDWFVRQVAAEDTDP	38.8	38.0	37.8	1.0 1.0
H2CA-3-B10-IR	QCSFAVLHENFYDWFARQVSGVEGLA	41.9	38.9	38.0	1.0 1.0
H2CA-3-A12-IR	QGNMSALHENFYDWFVRQVSEADRVD	37.8	7.3	6.3	1.2 0.9
H2CA-3-A8-IR	VAYPALLHEQFYDWFVRQVSAVAGTT	36.8	22.5	19.2	1.2 0.9
	PDTINSQHKNFYDWFVRQVSGVGTSS				

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FIG. 1K-2

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Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR
Parental	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
H2CA-4-F11-IR	VTFTSAVFHENFYDFVFRQVSVXXXXX	29.8	17.5	16.3	1.1	0.9
H2CA-4-E10-IR	TYKARFLHENFYDFWNRQVSYFGRV	37.7	2.2	18.1	0.1	8.2
H2CA-4-G3-IR	QRLSLHEQFYDFWFGVQVSPLAGG	31.2	4.4	18.8	0.2	4.3
H2CA-3-A11-IR	GGKVNTHEDFYGFVQVSGVGS DR	36.1	13.4	25.7	0.5	1.9
H2CA-4-F8-IR	LVGDAPFHEDFYDFWFRQVFGCCQEQ	35.6	12.1	22.0	0.5	1.8
H2CA-4-G4-IR	TGAEVSFHENFYDFWFRQVSSWLD RD	36.0	21.1	33.5	0.6	1.6
H2CA-4-F4-IR	QPHSSRLHESFYDFWFRQVWPYALDR	37.1	23.3	34.3	0.7	1.5
H2CA-4-H10-IR	SRALAAVHEQFYDFWFRQVSGLDWGY	39.8	25.0	35.6	0.7	1.4
H2CA-4-F1-IR	QPKDGTLHENFYDFWFRQVSSSGWVG	33.5	5.1	6.6	0.8	1.3
H2CA-3-D5-IR	RGRLLQLHEDFYDFWFLRQVSGMGGS	36.1	19.6	25.1	0.8	1.3
H2CA-4-E11-IR	QRGAPKSDENFYDFWFRQVLRFGEND	39.3	24.3	31.9	0.8	1.3
H2CA-3-B6-IR	AARTSLFHEDFYDFWFRQVQEGMWG	8.2	2.6	3.2	0.8	1.2
H2CA-3-A9-IR	GTSNHSLHENFYDFWFRQLSSVQSSG	35.9	9.9	12.1	0.8	1.2
H2CA-4-H5-IR	VSHVHLFHENFYDFWFRQLAAEGFSG	37.3	30.1	36.2	0.8	1.2
H2CA-3-C9-IR	GRQDSGLHEHFYDFWFRQVQGEVALG	38.6	35.4	37.3	1.0	1.1
H2CA-3-A10-IR	SNDERQFHETFYDFWFRQVSA DGADR	29.3	5.1	5.6	0.9	1.1
H2CA-3-A3-IR	LSTEQRFEHEFYDFWFRQVSTSGGT	37.2	16.9	19.1	0.9	1.1
H2CA-4-G8-IR	SLSRQFHENFYDFWFRQVSELEGV	29.2	28.6	32.2	0.9	1.1
H2CA-4-G9-IR	IPGRSLHENFYDFWFRQVSPGGSA	32.4	29.1	31.6	0.9	1.1
H2CA-4-G10-IR	TQKAQSLDEKFYDFWFRQVSGGLTG	36.1	34.4	36.4	0.9	1.1
H2CA-4-H7-IR	VSQLSDFHENFYDFWFRQVIAQAEWT	34.2	35.5	37.7	0.9	1.1
H2CA-4-F9-IR	NGTSQALHQNFXDFWFAQQISGSEPGP	37.0	36.0	40.0	0.9	1.1
H2CA-4-F7-IR	VGQSVTFHGDFYDFWFRQVSGSQEFG	37.5	36.7	39.5	0.9	1.1
H2CA-3-D10-IR	TI DHHPLHEQFYDFWFRQVSDLESIG	37.7	37.6	39.9	0.9	1.1
H2CA-3-B1-IR	PNVGYAFHENFYDFWFRQVSIIEKAG	18.7	3.6	3.5	1.0	1.0
H2CA-3-A5-IR	SRGSGVFHESFYDFWFRQVSEWIFQG	26.5	21.4	21.5	1.0	1.0
H2CA-4-F10-IR	QPVSGSVHERFYDFWFRQVSGSAGGG	32.9	22.9	22.4	1.0	1.0
	ASQLPPVYENFYDFWFRQVSLDAQRE	26.6	27.7	28.5	1.0	1.0

FIG. 1K-1

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Ratios over Background		Comparisons	
E-Tag	IGFsR	IGFR/IR	IR/IGFR
--	--	--	--
23.8	10.7	--	--
14.3	10.5	--	--
24.0	10.0	--	--
15.8	9.3	--	--
19.6	4.9	--	--
11.5	4.5	--	--
18.4	3.5	--	--
22.5	2.9	--	--
22.7	2.1	--	--

Sequence
 GFREGNFDWEVAQVT
 GFREGDYIGWFEEAQT
 GFREGDFYAWFMAQVT
 GFREGNFYEWFLAQVT
 GFREGSFYDWFDAQVT
 GFREGNFYDQFVAQVT
 GFREGHFYEWFAAQVT
 GFREGNFYEWFEVAQVT
 GFREGKFYDWFVAQVT
 GFREGMFDVQLLAQVT

Clone
 Design
 E4D-1B-B8-IGFR
 E4D-1-G7-IGFR
 E4D-1B-A11-IGFR
 E4D-1-C3-IGFR
 E4D-2-H1-IGFR
 E4D-1-C2-IGFR
 E4D-1B-A12-IGFR
 E4D-1B-A1-IGFR
 E4D-2-A3-IGFR

FIG. 1J-2

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
E4D-2-E7-IGFR	GFREGNFYDWFAQVT	20.8	22.8	--	--
E4D-2-C11-IGFR	GFREGDFYDWFAQVT	21.5	22.6	--	--
E4D-2-B1-IGFR	GFREGDFYDWFAQVT	22.0	22.5	--	--
E4D-2-D10-IGFR	GFREGGFYDWFAQVT	20.6	22.1	--	--
E4D-2-A9-IGFR	GFREGDFYDWFAQVT	17.4	21.5	--	--
E4D-2-E5-IGFR	GFREGDFYDWFAQVT	24.2	21.2	--	--
E4D-2-H9-IGFR	GFREGGFYDWFAQVT	19.1	20.7	--	--
E4D-1B-C4-IGFR	GFREGDFYDWFAQVT	24.3	20.5	--	--
E4D-2-E10-IGFR	GFREGNFYDWFAQVT	21.0	20.5	--	--
E4D-2-F4-IGFR	GFREGNFYDWFLAQVT	25.0	20.2	--	--
E4D-2-C10-IGFR	GFREGHFYDWFAQVT	22.8	20.1	--	--
E4D-3-D8-IGFR	GFREGQFYEWFAQVT	21.1	19.8	--	--
E4D-3-F9-IGFR	GFREGSFYEWFAQVT	22.6	19.7	--	--
E4D-1B-E5-IGFR	GFREGDFYDWFLAQVT	24.2	18.8	--	--
E4D-2-F3-IGFR	GFREGHFYDWFAQVT	23.6	18.0	--	--
E4D-3-D5-IGFR	GFREGQFYEWFAQVT	22.2	18.0	--	--
E4D-3-G10-IGFR	GFREGQFYDWFAQVT	22.1	17.6	--	--
E4D-2-F6-IGFR	GFREGQFYDWFAQVT	24.6	17.5	--	--
E4D-2-F7-IGFR	GFREGDFYQWFAAQT	19.0	17.5	--	--
E4D-3-B7-IGFR	GFREGNFYDWFAQVT	23.0	16.4	--	--
E4D-1B-C12-IGFR	GFREGSFYDWFAQVT	23.0	16.1	--	--
E4D-3-B1-IGFR	GFREGHFYEWFAQVT	21.6	16.0	--	--
E4D-2-E2-IGFR	GFREGDFYDWFAQVT	21.9	14.1	--	--
E4D-2-D1-IGFR	GFREGHFYDWFAQVT	24.5	13.2	--	--
E4D-1-D4-IGFR	GFREGYFYDWFKAQVT	18.9	12.4	--	--
E4D-1B-A10-IGFR	GFREGHFYDWFAQVT	23.9	10.8	--	--
E4D-1B-A3-IGFR	GFREGDFYDWFAQVT	22.2	10.8	--	--
E4D-1-B5-IGFR	GFREGTFYDWFAQVT	19.0	10.8	--	--

FIG. 1J-1

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
E4D β -4-F8-IR	GFREGNFYDWF Y FAQVT	--	--	--	--	--
E4D β -4-E12-IR	GFREGSFYDWF Y FAAQT	39.3	35.6	44.4	0.8	1.2
E4D β -4-H12-IR	GFREGSFYEWFD Y AAQT	40.2	27.8	33.4	0.8	1.2
E4D β -4-C9-IR	GFREGAFYDWF Y FAAQT	41.2	27.1	32.3	0.8	1.2
E4D β -4-H9-IR	GFREGQFYDWF Y FAAQT	38.0	22.5	27.6	0.8	1.2
E4D β -4-G9-IR	GFREGNFYDWF Y FAAQT	38.7	33.3	36.6	0.9	1.1
E4D β -4-F12-IR	GFREGDFYDWF Y FAAQT	10.9	4.9	5.6	0.9	1.1
E4D β -4-F9-IR	GFREGSFYEWFE Y AAQT	14.8	5.9	6.1	1.0	1.0
E4D β -4-F7-IR	GFREGGFYDWF Y LAQVT	39.3	31.3	28.3	1.1	0.9
E4D β -4-B7-IR	GFREGGFYAWFE Y AAQT	31.0	22.2	19.5	1.1	0.9
	GFREGGFYEW Y ?AQVT	--	--	--	--	--

FIG. 11-3

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
E4D α -4-H5-IR	GFREGNFYDNFVAQVT	47.2	36.0	14.7	2.4 0.4
E4D α -1-B12-IR	GFREGSFYEWFOAQVT	47.6	33.4	13.8	2.4 0.4
E4D α -4-G2-IR	GFREGNFYDNFVAQVT	23.4	20.4	8.6	2.4 0.4
E4D α -3-F9-IR	GFREGDFYDNFVAQVT	36.2	15.6	6.3	2.5 0.4
E4D α -4-G6-IR	GFREGDFYQWFFVAQVT	26.0	4.9	2.0	2.5 0.4
E4D α -4-H9-IR	GFREGGFYDNFVAQVT	47.8	24.8	9.5	2.6 0.4
E4D α -2-C10-IR	GFREGDFYGNFVAQVT	42.4	23.2	9.0	2.6 0.4
E4D α -1-B2-IR	GFREGVFYDNFVAQVT	39.4	18.7	7.2	2.6 0.4
E4D α -3-F12-IR	GFREGGFYEWFOAQVT	38.9	16.6	5.6	3.0 0.3
E4D α -2-D11-IR	GFREGSFYDNFVAQVT	40.2	11.1	3.3	3.4 0.3
E4D α -4-H2-IR	GFREGNFYEWFOAQVT	37.8	33.9	8.2	4.1 0.2
E4D β -4-A12-IR	GFREGKFYDNFLAQVT	41.1	8.3	28.7	0.3 3.5
E4D β -4-A10-IR	GFREGGFYEWFFVAQVT	5.8	1.2	2.4	0.5 2.0
E4D β -4-E10-IR	GFREGGFYDNFVAQVT	9.6	1.2	2.2	0.5 1.8
E4D β -4-B11-IR	GFREGTFYDNFVAQVT	36.1	15.2	26.9	0.6 1.8
E4D β -4-C10-IR	GFREGGFYEWFAAQVT	27.8	13.3	23.7	0.6 1.8
E4D β -4-E8-IR	GFREGDFYEWFEAQVT	28.7	16.7	28.2	0.6 1.7
E4D β -4-G7-IR	GFREGHFYDNFVAQVT	30.9	14.7	24.7	0.6 1.7
E4D β -4-C8-IR	GFREGGFYDNFVAQVT	35.5	22.5	32.9	0.7 1.5
E4D β -4-A8-IR	GFREGSFYDNFVAQVT	31.2	14.5	22.2	0.7 1.5
E4D β -4-A9-IR	GFREGSFYDNFVAQVT	35.8	9.0	13.1	0.7 1.5
E4D β -4-G11-IR	GFREGTFYDNFVAQVT	28.9	9.7	13.6	0.7 1.4
E4D β -4-B9-IR	GFREGNFYEWFFAQVT	27.2	9.1	12.5	0.7 1.4
E4D β -4-F10-IR	GFREGSFYNNFVAQVT	7.7	1.5	2.1	0.7 1.4
E4D β -4-D12-IR	GFREGNFYDNFVAQVT	41.1	27.2	36.1	0.8 1.3
E4D β -4-B8-IR	GFREGDFYDNFVAQVT	35.9	27.0	35.2	0.8 1.3
E4D β -4-G10-IR	GFREGAFYDNFVAQVT	38.5	25.5	33.7	0.8 1.3
E4D β -4-D9-IR	GFREGSFYDNFVAQVT	34.1	19.3	25.7	0.8 1.3

FIG. 11-2

Cl ne Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR	---
E4D α -1-B8-IR	GFREGNFYDNFVAQVT	40.7	1.0	12.3	0.1	12.3	---
E4D α -3-E5-IR	GFREGQRYWVFVAQVT	39.6	2.0	1.5	1.3	0.8	---
E4D α -1-A1-IR	GFREGYFYDNFLAQVT	48.7	44.9	31.4	1.4	0.7	---
E4D α -2-D9-IR	GFREGDFYENFVAQVT	22.9	3.3	2.4	1.4	0.7	---
E4D α -1-B3-IR	GFREGQFYENFVAQVT	41.8	38.6	26.5	1.5	0.7	---
E4D α -1-A6-IR	GFREGTFYDNFVAQVT	56.3	51.2	32.6	1.6	0.6	---
E4D α -1-A10-IR	GFREGNFYDNFVAQVT	48.9	42.2	26.5	1.6	0.6	---
E4D α -1-A8-IR	GFREGAFYDNFVAQVT	46.9	41.5	26.2	1.6	0.6	---
E4D α -1-B1-IR	GFREGAFYDNFVAQVT	44.1	31.1	19.7	1.6	0.6	---
E4D α -2-C9-IR	GFREGKFYQNFVAQVT	34.0	8.1	4.8	1.7	0.6	---
E4D α -1-A3-IR	GFREGDFYDNFVAQVT	45.3	40.3	22.5	1.8	0.6	---
E4D α -1-A9-IR	GFREGTFYENFVAQVT	46.9	41.0	22.5	1.8	0.5	---
E4D α -3-F3-IR	GFREGNFYDNFVAQVT	37.2	14.1	8.0	1.8	0.6	---
E4D α -2-D3-IR	GFREGQFYENFLAQVT	35.1	16.3	8.7	1.9	0.5	---
E4D α -2-D6-IR	GFREGQFYDNFLAQVT	33.2	5.6	2.8	2.0	0.5	---
E4D α -3-F10-IR	GFREGDFYDNFVAQVT	27.8	4.5	2.3	2.0	0.5	---
E4D α -2-D5-IR	GFREGQFYDNFVAQVT	43.8	23.8	11.4	2.1	0.5	---
E4D α -3-F4-IR	GFREGYFYENFVAQVT	25.9	7.6	3.7	2.1	0.5	---
E4D α -3-E3-IR	GFREGDFYQNFVAQVT	34.6	4.0	1.9	2.1	0.5	---
E4D α -3-F8-IR	GFREGSFGNFVAQVT	20.9	16.0	7.4	2.2	0.5	---
E4D α -2-C1-IR	GFREGSFGNFVAQVT	43.1	11.6	5.0	2.3	0.4	---
E4D α -1-B4-IR	GFREGQFYDNFVAQVT	45.3	6.6	2.9	2.3	0.4	---

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FIG. 11-1

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Clone	Parental/Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IGF _s R	IGF _s R/IR	IR/IGFR
A6L-4-F8-IGFR		YRGLVLGRISDGAGKVASVSPVRIQKQVAVNFYDWFV	19	4	--	--
A6L-2-G9-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	26	28	--	--
A6L-4-E7-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	39	22	--	--
A6L-4-G10-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	23	22	--	--
A6L-2-E9-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	19	22	--	--
A6L-2-D6-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	38	21	--	--
A6L-3-H12-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	34	21	--	--
A6L-4-A7-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	24	21	--	--
A6L-4-B8-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	20	20	--	--
A6L-4-G7-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	20	19	--	--
A6L-2-D9-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	19	19	--	--
A6L-4-F7-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	38	18	--	--
A6L-4-E12-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	18	18	--	--
A6L-4-H7-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	15	13	--	--
A6L-4-H12-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	14	13	--	--
A6L-2-A4-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	13	12	--	--
A6L-3-D10-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	17	4	--	--
A6L-2-F6-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	16	4	--	--
A6L-2-B11-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	15	4	--	--
A6L-1-B7-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	26	3	--	--
A6L-1-D8-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	23	3	--	--
A6L-0-A11-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	23	3	--	--
A6L-3-B7-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	19	3	--	--
A6L-1-G7-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	9	3	--	--
A6L-1-B9-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	20	2	--	--
A6L-1-C9-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	18	2	--	--
A6L-0-G10-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	18	2	--	--
A6L-1-G8-IGFR		YRGLVLGRISDVAGKACDPSARIQKQVLPVNFYDWFV	15	2	--	--

FIG. 1H

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Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
A6L-4-H9-IR		YRGMLVLGRISDAGKIVASEPPARIGQKVFAGNFYDWFV	19.0	4.0	--	--	--
A6L-4-E1-IR		YRAMLVLRISDVAGIVDSEPTTRIGQKVFAGNFYDWFV	37.5	27.3	27.3	1.0	1.0
A6L-3-A5-IR		YRGMLVLGRISQAGNVASEPSSRIGQKVFAGNFYDWFV	35.4	32.6	31.4	1.0	1.0
A6L-4-G4-IR		YRGMLVLGRISDAGKIVDYEPPARIGQKVFAGNFYDWFV	38.3	34.6	35.5	1.0	1.0
A6L-4-H2-IR		YRGMLGLGISAGAGIVASEPPARVQKVFAGNFYDWFV	30.4	17.7	15.2	1.2	0.9
A6L-4-E6-IR		YRGILFQGRIPDGAGKIVASEPTTRIGERVEAVNFYDWFV	36.1	4.2	3.6	1.1	0.9
A6L-4-H5-IR		QGGMPVLGRISDAGKIVAFEPARIGQKVFAGNFYDWFV	28.6	24.1	22.7	1.1	0.9
A6L-4-H3-IR		YRGMLVLGRISQDAGKIVASEPPARIGQKVFAGNFYDWFV	37.2	24.6	23.1	1.1	0.9
A6L-4-E5-IR		QRGMLVLGVSDGAGKIVASDPPASIGQNVFAVNFYDWFV	37.1	9.1	7.2	1.3	0.8
A6L-3-C5-IR		YPGMLILDRISDGASKVSEPPASIGQKVFAGNFYDWFV	42.1	30.6	24.4	1.3	0.8
A6L-4-G6-IR		YRGMLVLDLRISDGAGKIVASEQPARIGQEVAVNFYDWFV	42.2	21.9	17.5	1.2	0.8
A6L-3-D4-IR		YRGMLDLGRISGGVGKIVASEPPARIGQKVFAGNFYDWFV	29.8	4.3	2.8	1.5	0.7
A6L-3-A7-IR		QRGMLVLGRISDAGKIVASEKVFVNFYDWFV	39.9	12.4	8.4	1.5	0.7
A6L-3-A6-IR		QRGMLVLGRVSDGAGKIVDSAPPARIGQKVFAGNFYDWFV	31.0	21.2	14.0	1.5	0.7
A6L-4-E7-IR		QRGMLVLGRMSDGAGKIVAFEPARIGQKVFAGNFYDWFV	25.5	12.3	8.8	1.4	0.7
A6L-3-C6-IR		QRTIVLGRISDAGKAASEPPARIGQKVFAGNFYDWFV	38.4	12.5	7.1	1.7	0.6
A6L-4-F5-IR		QRGMLVLDLRISDAGKIVASEPPARIGQKVFAGNFYDWFV	28.8	10.9	6.7	1.6	0.6
A6L-3-B7-IR		QRGMLVLGRISDAGKIVASEPPARIGKVFAGNFYDWFV	33.8	6.3	4.1	1.5	0.6
A6L-4-F4-IR		QRGILVRGRISDAGKIVGSEPPARSGEKVFAGNFYDWFV	27.6	9.4	5.0	1.9	0.5
A6L-4-E3-IR		QLGMVVLGRISDGSKAASEPPARIQKVFAGNFYDWFV	38.9	17.6	9.4	1.9	0.5
A6L-0-E6-IR		QRGMLVLGRISDGDGKIVASEPPARIGQKVFAGNFYDWFV	38.0	6.9	3.8	1.8	0.5
A6L-0-E4-IR		YRGMLVLRSSDGAGKIVAFERPARIGQKVFAGNFYDWFV	31.0	31.0	1.8	17.0	0.1
A6L-0-H3-IR		YRGMLVLGRISDGAG#VASEPPARIGKVFAGNFYDWFV	26.0	16.0	1.3	13.0	0.1
		YRGMLVLGRISGGAGKAAERPARIGQKVFAGNFYDWFV	27.0	26.0	2.0	13.0	0.1

FIG. 1G-2

Cl ne	Parental/Design	Sequence	Ratios over Background		Comparisons		
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
A6L-3-D1-IR		<u>YR</u> G <u>M</u> L <u>V</u> L <u>G</u> R <u>I</u> S <u>D</u> G <u>A</u> G <u>K</u> V <u>A</u> S <u>E</u> P <u>P</u> A <u>R</u> I <u>G</u> O <u>K</u> V <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	19.0	.4.0	--	--	--
A6L-4-H7-IR		<u>Q</u> R <u>G</u> M <u>L</u> V <u>R</u> G <u>R</u> I <u>S</u> H <u>G</u> A <u>G</u> K <u>I</u> A <u>Y</u> E <u>P</u> P <u>D</u> C <u>L</u> G <u>O</u> K <u>A</u> C <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	22.6	19.8	26.5	0.7	1.3
A6L-4-H4-IR		<u>Q</u> R <u>G</u> M <u>L</u> L <u>L</u> G <u>R</u> I <u>S</u> D <u>D</u> A <u>G</u> K <u>V</u> A <u>S</u> E <u>P</u> S <u>A</u> R <u>R</u> G <u>O</u> K <u>V</u> F <u>A</u> E <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	37.5	3.5	4.2	0.8	1.2
A6L-4-E4-IR		<u>Y</u> R <u>G</u> I <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> E <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> E <u>P</u> A <u>R</u> I <u>G</u> O <u>K</u> V <u>F</u> A <u>D</u> F <u>Y</u> D <u>W</u> F <u>V</u>	38.5	21.1	25.8	0.8	1.2
A6L-4-G7-IR		<u>Q</u> R <u>G</u> M <u>L</u> A <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> G <u>I</u> G <u>O</u> K <u>V</u> F <u>A</u> E <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	38.1	5.4	6.0	0.9	1.1
A6L-3-C3-IR		<u>F</u> R <u>G</u> R <u>L</u> V <u>L</u> G <u>H</u> F <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> G <u>S</u> E <u>P</u> A <u>R</u> I <u>G</u> O <u>K</u> V <u>F</u> D <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	38.6	16.2	18.5	0.9	1.1
A6L-3-B6-IR		<u>Y</u> R <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> E <u>V</u> F <u>A</u> D <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	34.7	21.8	23.1	0.9	1.1
A6L-4-G11-IR		<u>Y</u> R <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> E <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> E <u>V</u> F <u>A</u> L <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	33.1	27.8	30.3	0.9	1.1
A6L-4-G12-IR		<u>V</u> P <u>W</u> Y <u>A</u> G <u>S</u> G <u>S</u> S <u>D</u> G <u>A</u> G <u>K</u> V <u>A</u> S <u>E</u> P <u>P</u> A <u>R</u> I <u>D</u> O <u>K</u> V <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	27.6	2.0	2.0	1.0	1.0
A6L-3-A10-IR		<u>Y</u> R <u>G</u> O <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> Y <u>G</u> A <u>G</u> K <u>V</u> G <u>C</u> D <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> K <u>D</u> W <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	32.0	2.3	2.3	1.0	1.0
A6L-4-E12-IR		<u>Q</u> R <u>G</u> L <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> N <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> G <u>I</u> G <u>O</u> E <u>V</u> F <u>P</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	21.1	2.4	2.4	1.0	1.0
A6L-4-E10-IR		<u>Q</u> R <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>A</u> E <u>P</u> P <u>D</u> C <u>L</u> G <u>O</u> K <u>V</u> C <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	3.1	2.4	2.4	1.0	1.0
A6L-4-G8-IR		<u>Q</u> R <u>G</u> M <u>R</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> E <u>L</u> P <u>P</u> R <u>I</u> G <u>O</u> K <u>D</u> F <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	30.1	3.8	3.8	1.0	1.0
A6L-3-C12-IR		<u>Q</u> R <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>Y</u> E <u>A</u> P <u>A</u> R <u>I</u> G <u>O</u> T <u>V</u> F <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	37.9	4.7	4.7	1.0	1.0
A6L-4-H11-IR		<u>Q</u> P <u>W</u> C <u>A</u> G <u>S</u> G <u>R</u> I <u>Y</u> D <u>G</u> A <u>C</u> K <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> H <u>I</u> G <u>O</u> E <u>V</u> A <u>V</u> N <u>F</u> Y <u>D</u> W <u>F</u> V	29.5	5.7	5.7	1.0	1.0
A6L-4-F10-IR		<u>Q</u> R <u>G</u> M <u>L</u> V <u>L</u> D <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> G <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> N <u>V</u> L <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	35.4	9.6	9.6	1.0	1.0
A6L-4-E9-IR		<u>Y</u> R <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> T <u>G</u> K <u>V</u> A <u>S</u> Q <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> K <u>V</u> F <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	31.6	10.5	10.5	1.0	1.0
A6L-4-H8-IR		<u>Y</u> R <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> V <u>P</u> P <u>A</u> H <u>I</u> G <u>O</u> K <u>V</u> F <u>A</u> E <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	39.8	12.9	12.9	1.0	1.0
A6L-3-A11-IR		<u>Q</u> H <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> S <u>E</u> P <u>P</u> A <u>R</u> I <u>G</u> H <u>K</u> V <u>F</u> D <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	38.2	14.6	14.6	1.0	1.0
A6L-4-F9-IR		<u>Y</u> S <u>G</u> Y <u>A</u> G <u>S</u> G <u>S</u> F <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> R <u>I</u> S <u>Q</u> E <u>V</u> L <u>A</u> D <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	29.0	17.5	17.5	1.0	1.0
A6L-4-G2-IR		<u>Y</u> R <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> K <u>V</u> S <u>A</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	35.7	18.4	18.4	1.0	1.0
A6L-4-E8-IR		<u>Y</u> H <u>G</u> K <u>L</u> D <u>L</u> G <u>R</u> I <u>S</u> V <u>G</u> V <u>G</u> K <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> K <u>V</u> F <u>A</u> D <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	29.5	21.4	20.7	1.0	1.0
A6L-4-H10-IR		<u>Y</u> R <u>G</u> A <u>G</u> S <u>G</u> V <u>G</u> S <u>L</u> T <u>V</u> A <u>G</u> K <u>V</u> A <u>S</u> D <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> K <u>V</u> F <u>A</u> D <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	28.7	21.6	21.6	1.0	1.0
A6L-4-G9-IR		<u>H</u> R <u>G</u> M <u>L</u> V <u>L</u> G <u>R</u> I <u>S</u> E <u>G</u> A <u>G</u> N <u>V</u> D <u>E</u> P <u>P</u> P <u>A</u> R <u>I</u> G <u>O</u> N <u>V</u> E <u>A</u> G <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	30.0	22.1	22.1	1.0	1.0
A6L-4-F7-IR		<u>Q</u> R <u>G</u> M <u>P</u> V <u>L</u> G <u>R</u> I <u>S</u> D <u>G</u> A <u>G</u> K <u>V</u> G <u>S</u> E <u>P</u> P <u>A</u> R <u>I</u> A <u>R</u> K <u>V</u> F <u>P</u> V <u>N</u> F <u>Y</u> D <u>W</u> F <u>I</u>	37.1	22.6	22.6	1.0	1.0
A6L-4-E11-IR		<u>Q</u> G <u>L</u> L <u>V</u> T <u>G</u> R <u>I</u> S <u>D</u> G <u>A</u> G <u>K</u> V <u>A</u> S <u>E</u> P <u>P</u> G <u>I</u> G <u>O</u> K <u>V</u> F <u>A</u> G <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	28.6	23.6	24.4	1.0	1.0
		<u>Y</u> P <u>W</u> Y <u>G</u> S <u>G</u> T <u>I</u> D <u>G</u> A <u>G</u> K <u>V</u> A <u>S</u> E <u>P</u> P <u>A</u> R <u>I</u> D <u>O</u> Q <u>V</u> E <u>A</u> G <u>N</u> F <u>Y</u> D <u>W</u> F <u>V</u>	38.4	26.5	26.5	1.0	1.0

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FIG. 1G-1

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Ratios over Background		Comparisons				
Clone	Design	E-Tag	IGF ₅ R	IR	IGFR/IR	IR/IGFR
A6S-1-D5-IGFR	Sequence	--	--	--	--	--
A6S-1-A2-IGFR	XXXXXXXXXXNFYDWFVXXXX	25	5	--	--	--
A6S-3-E6-IGFR	RVSGMEDLGNFYDWFVROAQ	20	5	--	--	--
A6S-1-G3-IGFR	KDPVTVSQGRNFYDWFVVIQ	20	5	--	--	--
A6S-3-G4-IGFR	DARDHGVWMSNFYDWFVAQVS	19	5	--	--	--
A6S-3-H8-IGFR	VATVHVGGGMNFYDWFVAQVG	21	4	--	--	--
A6S-3-E3-IGFR	CADPGACSSLNFYDWFVQMRG	20	4	--	--	--
A6S-3-D9-IGFR	NPTSVQQYGVNFYDWFVNVL	19	4	--	--	--
A6S-2-A1-IGFR	RPSLPEVRPGNFYDWFVQSVR	17	4	--	--	--
A6S-1-H4-IGFR	SLQGADEFQGNFYDWFVSELA	31	3	--	--	--
A6S-3-C1-IGFR	LSSRGVTRNFYDWFVAQVV	18	3	--	--	--
A6S-3-B10-IGFR	HKSWTMSPLNFYDWFVAQVE	17	3	--	--	--
	RPVIGGGGTRNFYDWFVAQMI	16	3	--	--	--
	YDQDPPYWGILNFYDWFVREVA			--	--	--

FIG. 1F-3

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Ratios over Background		Comparisons				
Clone	Design	Sequence	E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
A6S-4-A6-IGFR		XXXXXXXXXXXXNFYDFVXXXX	--	--	--	--
A6S-4-A3-IGFR		HVEHMAVGDNFYDFVQQLR	21	23	--	--
A6S-4-D3-IGFR		RGMTGMVGRGNFYDFVQQLR	21	23	--	--
A6S-3-E10-IGFR		GLRSEQGNRLNFYDFVQQLA	20	23	--	--
A6S-4-D1-IGFR		RVREKLPRPENFYDFVQQLH	23	22	--	--
A6S-4-B2-IGFR		SNPSRQDASVNFYDFVREVA	22	22	--	--
A6S-4-A2-IGFR		QSVDLSPDSNFYDFVEVL	21	22	--	--
A6S-4-A5-IGFR		IGGQGHQDGNFYDFVEALA	20	22	--	--
A6S-4-C1-IGFR		VEVQRHKKDNFYDFVKQID	19	22	--	--
A6S-4-B1-IGFR		CWARPCGDAANFYDFVQQLS	16	22	--	--
A6S-4-B4-IGFR		RHERGKEGPGNFYDFVQQLV	19	21	--	--
A6S-4-D4-IGFR		ERSPRPALASNFYDFVQQLV	19	21	--	--
A6S-3-F8-IGFR		IARMRETFQPNFYDFVQQLA	18	21	--	--
A6S-3-H9-IGFR		GRGQGLKRPDNFYDFVAAAK	25	20	--	--
A6S-3-G2-IGFR		YSIEVQDNENFYDFVFSQLG	23	20	--	--
A6S-4-H2-IGFR		TWMWEERKQDNFYDFVQQLK	21	20	--	--
A6S-4-A3-IGFR		VTFTSAVFHENFYDFVFRQVS	19	20	--	--
A6S-3-G10-IGFR		LAINDLVTHKNFYDFVQQLR	18	20	--	--
A6S-3-E5-IGFR		GAVGLAEAGPNFYDFVQQLV	24	19	--	--
A6S-3-H2-IGFR		RYRGERHDGRNFYDFVEQVN	21	19	--	--
A6S-3-G3-IGFR		QGAEGRLSEGNFYDFVQAVS	21	19	--	--
A6S-4-H1-IGFR		PRLHMGSDMGDFYDFVQQLA	21	18	--	--
A6S-4-G1-IGFR		IVAGARHSEVNFYDFVQQLR	18	18	--	--
A6S-4-A1-IGFR		AELVGAGVRGNFYDFVQQLV	16	16	--	--
A6S-2-F1-IGFR		DSSRLWLGERNFYDFVQQLS	17	12	--	--
A6S-2-G1-IGFR		VGQVGRYVRSNFYDFVQQLM	30	8	--	--
A6S-1-C5-IGFR		RPQLVESGSKNFYDFVQQLV	30	8	--	--
A6S-2-B2-IGFR		RIHNQTERGGNFYDFVHQLV	27	7	--	--
		EMYGDTSERVNFYDFVSAIQ	30	5	--	--

FIG. 1F-2

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Ratios over Background		Comparisons	
E-Tag	IGFsR	IGFR/IR	IR/IGFR
--	--	--	--
27	32	--	--
36	30	--	--
35	30	--	--
26	30	--	--
26	30	--	--
21	29	--	--
40	28	--	--
36	28	--	--
25	28	--	--
24	28	--	--
20	28	--	--
20	28	--	--
42	27	--	--
24	26	--	--
23	26	--	--
19	26	--	--
18	26	--	--
37	25	--	--
25	25	--	--
20	25	--	--
25	24	--	--
22	24	--	--
22	24	--	--
21	24	--	--
19	24	--	--
18	24	--	--
17	24	--	--
30	23	--	--

Clone	Sequence
<i>D sign</i>	XXXXXXXXXXNFYDFWVXXXX
A6S-4-E4-IGFR	ERSAAGFREGNFYDFWVQAQVN
A6S-2-D2-IGFR	RAERGSMDNSNFYDFWVQQLP
A6S-2-F2-IGFR	LAMSVASRPANFYDFWVQAQIV
A6S-4-F3-IGFR	HNSSSPMRGTGNFYDFWVQELR
A6S-4-G4-IGFR	SALSGPVQPINFYDFWVFTGM
A6S-4-G3-IGFR	GAQAIREIHHNFYDFWVQAQVT
A6S-2-H2-IGFR	RGQRESDSGTNFYDFWVFGAIR
A6S-2-E3-IGFR	VQEGLSGMEGNFYDFWVDQLF
A6S-4-C6-IGFR	RLDRSSTSGVNFYDFWVQVVG
A6S-4-F5-IGFR	GSQHSGREPHNFYDFWVQVVG
A6S-4-H3-IGFR	GRGDQRHETTNFYDFWVRELQ
A6S-4-H4-IGFR	PRMVEKPSEDNFYDFWVFTQLS
A6S-2-H1-IGFR	RVGIQVDPHTNFYDFWVFIQLT
A6S-4-E6-IGFR	RSSGGLLSQGNFYDFWVFSQLE
A6S-4-B6-IGFR	SDARQAGLQENFYDFWVFSQVR
A6S-4-D2-IGFR	PPYRSSRLGENFYDFWVFMQVR
A6S-4-G5-IGFR	QEVTRTRDDKNFYDFWVFSQIF
A6S-2-A3-IGFR	SRAPYGSTAGNFYDFWVQAVS
A6S-4-E2-IGFR	?DQSVSSKGNFYDFWVQQMT
A6S-4-G6-IGFR	RLMGGAEPQNFYDFWVREVA
A6S-4-G2-IGFR	SAGHHMPRESNFYDFWVDQVW
A6S-4-D6-IGFR	LGAAETWDGINFYDFWVFKQVS
A6S-4-F4-IGFR	VGHSGVPPYPNFYDFWVFMQVS
A6S-4-C3-IGFR	VTMLDKGAQDNFYDFWVREVA
A6S-4-H5-IGFR	HHSPGNEHGYNFYDFWVFLQVA
A6S-4-H6-IGFR	GSIAQLIMRANFYDFWVEQTN
A6S-4-F6-IGFR	LKGSQQLSVNFYDFWVQQIK
A6S-3-H1-IGFR	PASNKNLSAENFYDFWVQQTR

FIG. 1F-1

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
A6S-4-G1-IR	XXXXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--	
A6S-1-A3-IR	NGVERAGTGDNFYDWFVAQLH	36.2	31.8	15.7	2.0	0.5	
A6S-3-F12-IR	PFAGKGDKTGNFYDWFVSLTG	39.9	12.6	6.0	2.1	0.5	
A6S-4-G2-IR	GMPQEXMDQVNFYDWFVAQVD	41.4	7.4	4.0	1.9	0.5	6 / 200
A6S-1-B1-IR	MGTPAVGDGANFYDWFVRQLG	26.7	7.0	3.5	2.0	0.5	
A6S-2-D11-IR	SKCKAWYGANNFYDWFVWQVD	30.6	3.7	1.9	1.9	0.5	
A6S-2-D1-IR	EAASLGSQDRNFYDWFVRQVV	48.4	37.4	13.5	2.8	0.4	
A6S-3-E2-IR	VERSASSQDGNFYDWFVQIR	37.8	30.6	12.0	2.6	0.4	
	TSEVQRRSQDNFYDWFVAQVA	33.1	24.7	9.8	2.5	0.4	

FIG. 1E-3

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF _s R	IR	IGF _s R/IR
A6S-3-E9-IR	XXXXXXXXXXNFYDWFVXXXX	36.6	9.0	8.9	1.0
A6S-3-E1-IR	TTCHPRGEDCNFYDWFVQLR	36.7	6.8	6.9	1.0
A6S-4-H12-IR	VRGNDSVLRANFYDWFVDQLS	46.3	6.1	5.8	1.1
A6S-2-D3-IR	TPRSQVRSDHNFYDWFVYQLA	37.0	5.3	5.1	1.0
A6S-3-E8-IR	ESLTGSRPDRNFYDWFVQOTS	42.7	5.2	5.1	1.0
A6S-1-A12-IR	PQSLTEVRTGNFYDWFVQLH	39.7	2.1	2.1	1.0
A6S-4-H3-IR	DVGMGRVKETNFYDWFVRQLI	18.6	3.1	2.9	1.1
A6S-3-F7-IR	GADDIRSLTNFYDWFVNQLS	46.2	2.3	2.1	1.1
A6S-2-D8-IR	GVSIOAGYKTNFYDWFVEAVR	31.2	2.0	1.7	1.2
A6S-3-F10-IR	VEGHRQMSVGNFYDWFVQIA	39.0	5.9	4.5	1.3
A6S-4-G11-IR	GSSLGRSGPGNFYDWFVDQLE	44.8	4.3	3.3	1.3
A6S-2-D2-IR	HRQQDVVRQGNFYDWFVQALE	33.5	3.6	2.7	1.3
A6S-4-G8-IR	QDTFLTAREGNFYDWFIRALE	11.1	2.5	1.9	1.3
A6S-4-H6-IR	EAIMREEGQANFYDWFVRQLE	22.4	2.4	1.9	1.3
A6S-2-D10-IR	VCDVSTGGGTNFYDWFVCQVG	41.3	2.1	1.7	1.2
A6S-3-F4-IR	POPRASSTPLNFYDWFVQATG	37.0	13.5	9.9	1.4
A6S-4-G9-IR	GVSRGSGDPNFYDWFVMQLR	36.2	11.8	7.8	1.5
A6S-3-F5-IR	GPGRHDSRGNFYDWFVEQLA	48.1	7.2	4.8	1.5
A6S-4-H1-IR	ERFALEVQGSNFYDWFVRQVI	18.3	3.6	2.6	1.4
A6S-3-F6-IR	NLKSSATVGGNFYDWFVEQL	18.7	2.9	1.9	1.5
A6S-3-F11-IR	MEGPPAGGPLNFYDWFVAQVD	33.8	2.0	1.4	1.4
A6S-2-C6-IR	RLDVAGHRGGNFYDWFVKQLH	46.7	19.2	12.1	1.6
A6S-4-G4-IR	PWSDHEALNQNFYDWFVSQVL	36.9	18.2	10.7	1.7
A6S-4-G12-IR	EDRLNGESTNFYDWFVRQLA	32.8	12.8	7.9	1.6
A6S-2-D7-IR	GKLVASTLDDNFYDWFVRQLS	33.2	12.0	7.1	1.7
A6S-4-G10-IR	SGPVVQTQGNFYDWFVHQLR	33.9	10.8	6.8	1.6
A6S-3-F9-IR	VDRAGPAGSDNFYDWFVAQLD	44.3	9.6	5.7	1.7
A6S-3-F2-IR	SLGRNDRPDENFYDWFVSQVQ	23.2	4.3	2.5	1.7
	RVMTATANAPMNFYDWFVQLQ				0.6

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FIG. 1E-2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
A6S-3-E12-IR	XXXXXXXXXXXXNFYDFVXXXX	26.2	1.3	8.0	0.2
A6S-2-C1-IR	GRVDWLQARNANFYDFVFAELG	41.2	1.3	7.0	0.2
A6S-1-A7-IR	RMFESTGAPQNFYDFVQEW	47.2	2.3	11.1	0.2
A6S-2-C8-IR	HHTQGLQQRNFYDFVFNELR	44.9	1.5	5.5	0.3
A6S-3-E10-IR	MHRMQHDGTSNFYDFVFLQWA	46.9	1.6	5.0	0.3
A6S-2-D5-IR	AMHVVAQGGPNFYDFVREL	31.9	1.2	3.7	0.3
A6S-1-B2-IR	AIOMNGNLAFNFYDFVRELT	31.6	1.8	5.3	0.3
A6S-1-A4-IR	TDRKSVQEPNRFYDFVWAAR	43.3	3.6	9.2	0.4
A6S-4-G3-IR	PHGRGFAQSNFYDFVFTQEE	31.3	2.3	5.1	0.5
A6S-4-H8-IR	RLASASVPGQNFYDFVFDQLL	11.5	1.7	3.6	0.5
A6S-3-E11-IR	RQSEFSTLNSNFYDFVRELE	26.3	2.3	4.4	0.5
A6S-1-A1-IR	GQAQLSIRDVNFYDFVQQLV	36.9	3.7	6.5	0.6
A6S-2-C9-IR	MSEPAVGNGNFYDFVFAQLF	43.6	1.3	2.3	0.6
A6S-2-C4-IR	VGTGRARLDRNFYDFVFGQYS	34.5	5.6	9.6	0.6
A6S-4-H10-IR	SREAVQKRANFYDFVQQLS	39.2	4.4	6.9	0.6
A6S-4-G7-IR	LAQFAGSRNQNFYDFVFEQLG	19.1	1.4	2.2	0.6
A6S-4-H2-IR	GQEIFDQMGILNFYDFVRELD	25.5	2.6	3.9	0.7
A6S-2-C3-IR	RQPSQPPHGSNFYDFVFEAIN	31.1	1.6	2.4	0.7
A6S-2-C11-IR	LMQSLGSGSTNFYDFVQOMV	20.9	3.3	4.6	0.7
A6S-3-F3-IR	DQORSACDGTNFYDFVVCQLS	37.1	3.0	4.2	0.7
A6S-3-E5-IR	LDGTKACQVRNFYDFVVCQTE	31.6	2.5	3.5	0.7
A6S-1-B7-IR	PEARRTVVHSNFYDFVFAQLS	49.2	1.6	2.3	0.7
A6S-3-E7-IR	PWMLSVGIQDNFYDFVVGGLDS	37.2	5.0	6.3	0.8
A6S-4-G6-IR	ASHQRGGSSDNFYDFVFAQMR	16.8	3.1	4.0	0.8
A6S-2-C2-IR	TLERGEFSGNFYDFVFEQLH	29.7	2.4	3.1	0.8
A6S-3-F1-IR	DRQISGSVHGDFYDFVFSALG	29.7	2.3	3.0	0.8
A6S-2-C5-IR	DWDKLGSLSENFYDFVFDQLA	42.9	6.1	7.0	0.9
A6S-3-E4-IR	VRVVLNQSGRNFYDFVFIQLE	20.9	2.1	2.3	0.9
	MASWQSRTPDNFYDFVRELS				

FIG. 1E-1

Ratios over Background		Comparisons	
E-Tag	IGFsR	IGFR/IR	IR/IGFR
--	--	--	--
40.1	16.6	--	--
39.2	13.9	--	--
36.7	8.0	--	--
40.2	4.1	--	--

Sequence
 xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
 DPERMQSDVGFYEWFRRAAVG
 DIGSDGHGRRWDSFYRWFEM
 PFYQWFLDQSVGGSRGGGLR
 AVAPLSVRGRDSCGFYSWFSS

Clone
 Design
 R20-4-B9-IGFR
 R20-4-F8-IGFR
 R20-4-G12-IGFR
 R20-4-D10-IGFR

FIG. 1D

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Cl ne Design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGFsR --	IR --	IGFR/IR --
R20 α -3-20D3-IR	IGGQGHQDGNFYDWFVEALA	46.3	36.2	7.0	5.2
R20 α -3-20F1-IR	VFNCRSQQLDFYEWFEQAA	49.0	26.0	2.8	9.3
R20 α -3-20H1-IR	RVAGAIAPGLVSNKQDGLFYSWFRE	45.6	35.3	3.3	10.7
R20 α -3-20D1-IR	VLQARHGCDSDVDCFYEWFA	50.8	37.5	3.0	12.5
R20 β -4-B12-IR	GAFYRWFEALVGSERVPDV	41.9	2.9	5.7	0.5
R20 β -4-H3-IR	HEAFYDWFSAIVDGGYELMG	13.9	5.8	2.4	2.4
R20 β -4-D10-2-IR	RIGGGWARSEGFYEFVREL	21.5	7.3	2.9	2.5
R20 β -4-C8-IR	LPAGGA?GFA?RGFYEFES	44.9	31.1	9.6	3.2
R20 β -4-E7-IR	GHSWALVRHVDRRLFYEWFDL	45.0	18.8	5.9	3.2
R20 β -4-E7-2-IR	LGTSAQGQVGHRAFYQWFQS	45.0	18.8	5.9	3.2
R20 β -4-G3-IR	RGGGTFYEFESALRKHGAG	38.6	7.5	2.0	3.8
R20 β -4-H6-IR	NSSGQVVGLTIFYSWFASQV	14.8	7.6	2.0	3.8
R20 β -4-G11-IR	FYGFWSRQLSLTPRDDWGLP	39.4	7.5	1.9	3.9
R20 β -4-G8-IR	RMFYEFWSQMGAGPTEGSA	41.2	15.1	3.4	4.4
R20 β -4-H9-IR	IGGQGHQDGNFYDWFVEALA	43.1	8.8	2.0	4.4
R20 β -4-H8-IR	RDKPTDQEEQNWSFYEFWRH	47.9	43.7	9.3	4.7
R20 β -4-B8-IR	WSALLSVMDTGFYAWFDDAV	44.0	40.1	8.4	4.8
R20 β -4-E2-IR	SRDQTNFTFNSAGFYGWFER	16.3	13.9	2.4	5.8
R20 β -4-F4-IR	GVGTLTMSSDAFYTWIV	15.3	5.9	1.0	5.9
R20 β -4-A8-IR	IGGSFVEFYGFNFNDQV	43.3	36.0	6.0	6.0
R20 β -4-C4-IR	DIGSDGHGRRWDSFYRWFEW	17.3	26.8	4.3	6.2
R20 β -4-D7-IR	VLQARHGCDSDVDCFYEWFA	44.8	36.2	5.6	6.5
R20 β -4-D2-IR	DPERMQSDVGFYEFWFRAAVG	31.2	29.4	2.9	10.1

FIG. 1C

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Ratios over Background			Comparisons		
E-Tag	IGFsR	IR	IGF/IR	IR/IGF	
--	--	--	--	--	--
40.3	9.0	2.0	4.5	0.2	
60.4	12.9	2.0	6.5	0.2	
52.6	37.5	2.0	18.8	0.1	

[illegible]

FIG. 1A

FIG. 1B

Clone	Design	Sequence
R40-3-40B2-IR		IRDMHYVWQDRDRYINGVRQWYISDRYNPGSAFYRWIFD
R40-4-40B12-IR		RMGLQALAHYRKSAGPIFLSGSVIKGSEGDPPYAWFRLQ
R40-4-40G11-IR		MPVSLFRRVWDYRDGEHETLESHYVVPQAALDRLLFYSWFS

Clon design	Sequence
R40-3-D5-IGFR	PLYGGGIHLYPGTMGYVPFPQVKVLGDADKNFYDWFEM
R40-3-A6-IGFR	YRGMVLGRISDGAGKVASEPPARIGQKVFAYNFYDWFV
R40-X-R35-IGFR	SGCCRLLGRLRMFIVIVGWSGALVCQSAASAAGFYDWFV

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- 260. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 7.
- 261. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 8.
- 5 262. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 9.
- 263. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 10.

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251. The amino acid sequence according to claim 248 wherein the amino sequence binds to Site 2 of IGF-1R.
252. An amino acid sequence comprising the sequence $WX_{123}GYX_{124}WX_{125}X_{126}$ wherein X_{123} is proline, glycine, serine, arginine, alanine or leucine, X_{124} is any amino acid; X_{125} is a hydrophobic amino acid; and X_{126} is any amino acid.
253. The amino acid sequence according to claim 252 wherein X_{123} is proline and X_{125} is leucine or phenylalanine.
254. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 1.
255. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 2.
256. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 3.
257. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 4.
258. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 5.
259. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 6.

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245. The amino acid sequence according to claim 243 wherein the amino sequence binds to Site 1 of IR.
246. The amino acid sequence according to claim 243 wherein the amino sequence binds to Site 2 of IR.
- 5 247. A method of screening for a compound which binds to IGF-1R comprising:
- i) immobilizing IGF-1R, or a fragment thereof, on a surface;
 - ii) incubating the IGF-1R, or fragment thereof, with a known amount of labeled amino acid sequence of Formulas 1-9, or an amino acid sequence selected from Figure 10, which binds IGF-1R and a compound to be screened under
 - 10 conditions which provide for binding of the labeled amino acid sequence to bind to IGF-1R;
 - iii) measuring the amount of labeled amino acid sequence bound to IGF-1R;
 - iv) determining from the amount of bound labeled peptide
 - 15 whether the compound has competitively bound to IGF-1R.
248. An amino acid sequence capable of bind to Site 1 or Site 2 of IGF-1R identified by the method according to claim 247, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
249. The amino acid sequence according to claim 248 wherein the amino acid
- 20 sequence is an IGF agonist.
250. The amino acid sequence according to claim 248 wherein the amino sequence binds to Site 1 of IGF-1R.

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239. A method of treating a patient with an IGF sensitive tumor comprising administering to an individual in need of treatment a therapeutically effective amount of an amino acid sequence which is an IGF-1R antagonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
240. The method according to claim 239 wherein the amino acid sequence is expressed by a recombinant vector administered to the individual.
241. The method according to claim 239 wherein the amino acid sequence is administered to the individual as a polypeptide.
242. A method of screening for a compound which binds to IR comprising:
- i) immobilizing IR, or a fragment thereof, on a surface;
 - ii) incubating the IR, or fragment thereof, with a known amount of labeled amino acid sequence of Formulas 1-10, or an amino acid sequence selected from Figures 10-11, which binds IR and a compound to be screened under conditions which provide for binding of the labeled amino acid sequence to bind IR;
 - iii) measuring the amount of labeled amino acid sequence bound to IR;
 - iv) determining from the amount of bound labeled peptide whether the compound has competitively bound to IR.
243. An amino acid sequence capable of binding to Site 1 or Site 2 of IR identified by the method according to claim 242, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
244. The amino acid sequence according to claim 243 wherein the amino acid sequence is an IR agonist.

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231. The composition according to claim 230, wherein the peptide comprises the amino acid sequence NFYDWFV.
232. The pharmaceutical composition according to claim 230, wherein the peptide comprises the amino acid sequence QMKDIFYSLLASLAA.
- 5 233. A pharmaceutical composition comprising a amino acid sequence which binds specifically to IR receptor at Site 1 and is an insulin agonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof, and a pharmaceutically acceptable carrier.
- 10 234. The pharmaceutical composition according to claim 233, wherein the peptide comprises the amino acid sequence FYDWF.
235. The pharmaceutical composition according to claim 233, wherein the peptide comprises the amino acid sequence FYSLLASL.
- 15 236. A method of treating diabetes comprising administering to an individual in need of treatment a therapeutically effective amount of an amino acid sequence which binds IR at Site 1 and is an insulin agonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
237. The method according to claim 236 wherein the amino acid sequence is expressed by a recombinant vector administered to the individual.
- 20 238. The method according to claim 236 wherein the amino acid sequence is administered to the individual as a polypeptide.

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5
NSFYEALRMLSS,
SLNFYDALQLLA,
SSNFYQALMLLS,
SDGFYNAIELLS,
HETFYSMIRSLA,
HDPFYSMMKSL and
WSDFYSYFQGL.

- 10 224. A kit for identifying a compound which binds IGF-1 receptor, comprising a IGF-1 receptor and an amino acid sequence selected from Formulas 1-10, or the amino acid sequences of Figures 9-11, which bind to the receptor at Site 1 or Site 2.
225. The kit according to claim 224, wherein the amino acid sequence comprises the amino acid sequence FYDWF.
- 15 226. The kit according to claim 225, wherein the amino acid sequence comprises the amino acid sequence SAKNFYDWFVKK.
227. The kit according to claim 226 wherein the amino acid sequence comprises the amino acid sequence FYSLLASL.
228. The kit according to claim 227 wherein the amino acid sequence comprises the amino acid sequence QMKDIFYSLLASLAACK.
- 20 229. A kit for identifying a compound which binds IR comprising IR and an amino acid sequence selected from Formulas 1-10 or the amino acid sequences of Figures 9 and 11 which bind IR at Site 1 or Site 2.
- 25 230. A pharmaceutical composition comprising a amino acid sequence which binds specifically to IGF-1 receptor at Site 1 and is an IGF agonist, with the proviso that the amino acid sequence is not IGF-1, insulin, or fragments thereof, and a pharmaceutically acceptable carrier.

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AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENF
YDWFVRQVSAAAGAPVPYPDPLEPRAA,

AQPAMAFHENFYDWFVRQVSGGSGGSFHENFYDWFVRQVSAAAG
APVPYPDPLEPRAA,

5 AQPAMAFHENFYDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAA
AGAPVPYPDPLEPRAA and

AQPAMAFHENFYDWFVRQVSGGSGGSGGSGGSFHENFYDWFVRQV
SAAAGAPVPYPDPLEPRAA.

220. A nucleic acid sequence encoding amino acid sequence which binds to IR at
10 Site 1 and/or Site 2, with the proviso that the sequence is not insulin, IGF, or
fragments thereof.

221. The nucleic acid sequence according to claim 220 wherein the nucleic acid
sequence encodes for an amino acid sequence selected from the group
consisting of FYDWF, FYEWF, FHENFYDWF, FHENFYDWFVRQVSK,
15 DYKDVTFITSAVFHENFYDWFVRQVSKK, GRVDWLQRNANFYDWFV
AELG and APTFYAWFNQQT.

222. The nucleic acid sequence according to claim 220 wherein the nucleic acid
sequence encodes for an amino acid sequence selected from the group
consisting of DYKDFYDAIDQLVRGSARAGGTRDKK and
20 KDRAFYNGLRDLVGAVYGAWDKK.

223. The nucleic acid sequence according to claim 220 wherein the nucleic acid
sequence encodes for an amino acid sequence selected from the group
consisting of SFYEAIHQLLGV,

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215. The amino acid sequence according to claim 203 wherein the two amino acid sequences are connected by a peptide or non-peptide linker.
216. The amino acid sequence according to claim 215 wherein the linker is a peptide consisting of about 2 to about 16 amino acids.
- 5 217. The amino acid sequence according to claim 215 wherein the linker is a non-peptide.
218. The amino acid sequence according to claim 217 wherein the linker is dialdehyde.
219. The amino acid sequence according to claim 203 wherein the amino acid
10 sequence is selected from the group consisting of

DYKDDDDKFHENFYDWFVRQVSGSGGLDALDRLMRYGEERPSLA
AAGAP,

DYKDDDDKFHENFYDWFVRQVSGGSHLCVLEELFWGASLFGYCSG
AAAGAPVPYPDPLEPRAA,

15 DYKDDDDKFHENFYDWFVRQVSGGSGGSGGSHLCVLEELFWGASL
FGYCSGAAAGAPVPYPDPLEPRAA,

DYKDDDDKFHENFYDWFVRQVSGGSGGSGGSGGSHLCVLEELFWG
ASLFGYCSGAAAGAPVPYPDPLEPRAA,

20 AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAAGAPVP
YPDPLEPRAA,

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212. The amino acid sequence according to claim 211 wherein at least one of the sequences comprises LX₁₅, X₁₆, LLX₁₉YF.
213. The amino acid sequence according to claim 203 wherein at least one of the sequences comprises
- 5 X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀X₄₁
 wherein X₂₂, X₂₅, X₂₆, X₂₈, X₂₉, X₃₀, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈, X₄₀, and X₄₁ are any amino acid, X₂₃ is any hydrophobic amino acid; X₂₇ is a polar amino acid; X₃₁ is an aromatic amino acid; X₃₂ is a small amino acid, and wherein at least one cysteine is located at positions X₂₄ through X₂₇ and one
- 10 at X₃₉ or X₄₀; X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅
 X₅₆X₅₇X₅₈X₅₉ X₆₀ X₆₁ wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₈, X₆₀ and X₆₁ are any amino acid; X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids; X₄₇ and X₅₉ are cysteine; X₄₈ is a polar amino acid; and X₅₁, X₅₂ and X₅₇ are small amino acids; or X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂
- 15 X₇₃ X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ X₇₉ X₈₀ X₈₁ wherein X₆₂, X₆₅, X₆₆, X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀ and X₈₁ are any amino acid; X₆₃, X₇₀, and X₇₄ are hydrophobic amino acids; X₆₄ is a polar amino acid; X₆₇ and X₇₅ are aromatic amino acids; and X₇₂ and X₇₉ are cysteines.
214. The amino acid sequence according to claim 203 wherein at least one of the sequences comprises HX₈₂X₈₃X₈₄X₈₅X₈₆X₈₇X₈₈X₈₉X₉₀X₉₁X₉₂ herein X₈₂ is proline or alanine; X₈₃ is a small amino acid; X₈₄ is selected from the group consisting of leucine, serine and threonine; X₈₅ is a polar amino acid; X₈₆ is any amino acid; X₈₇ is an aliphatic amino acid; X₈₈, X₈₉, X₉₀ is any amino acid; and X₉₁ and X₉₂ are aliphatic amino acids or
- 25 X₁₀₄X₁₀₅X₁₀₆X₁₀₇X₁₀₈X₁₀₉X₁₁₀X₁₁₁X₁₁₂X₁₁₃X₁₁₄ wherein at least one of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan; wherein X₁₀₄ and X₁₁₄ are both small amino acids; wherein X₁₀₅ is any amino acid; and wherein at least one of X₁₀₄, X₁₀₅, X₁₀₆ and one of X₁₁₂ X₁₁₃ X₁₁₄ are cysteine residues.

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206. The amino acid sequence according to claim 203, wherein at least one of the sequences is selected from the group consisting of $X_1X_2X_3X_4X_5$ wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 may be any polar amino acid; $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids; and $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
207. The amino acid sequence according to claim 206, wherein at least one of the sequences is $X_1X_2X_3X_4X_5$ wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 may be any polar amino acid.
208. The amino acid sequence according to claim 206 wherein at least one of the sequences comprises FYX_3WF .
209. The amino acid sequence according to claim 206, wherein at least one of the sequences comprises $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids.
210. The amino acid sequence according to claim 209, wherein at least one of the sequences comprises $FYX_8X_9LX_{11}X_{12}L$.
211. The amino acid sequence according to claim 206, wherein at least one of the sequences comprises $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

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198. The amino acid sequence according to claim 197 comprising
ACVWPTYWNCG.
199. A method of providing insulin agonist activity to mammalian cells, said
method comprising administering to said cells an amino acid sequence
5 comprising DYKDLQCSWGVRIGWLAGLCPKK.
200. A method of modulating insulin activity in mammalian cells, said method
comprising administering to said cells an amino acid sequence comprising
an amino acid sequence selected from the group listed in Figures 9 through
11.
- 10 201. An amino acid sequence comprising DYKDLQCSWGVRIGWLAGLCPKK.
202. An amino acid sequence comprising an amino acid sequence selected from
the group listed in Figures 9 through 11.
203. An amino acid sequence comprising at least two amino acid sequences
which independently bind IR, with the proviso that at least one of the
15 sequences is not insulin or a fragment thereof.
204. The amino acid sequence according to claim 203 wherein the two amino
acid sequences bind to Site 1 of IR.
205. The amino acid sequence according to claim 203 wherein one amino acid
sequence binds to Site 1, and the other binds to Site 2 of IR.

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188. The amino acid sequence according to claim 187 wherein at least two of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan which are separated from each other by at least two amino acids.
- 5 189. The amino acid sequence according to claim 188 wherein the separating amino acids are selected from the group consisting of proline, threonine and tyrosine.
190. The amino acid sequence according to claim 189 wherein the amino acid sequence comprises WPTYW.
- 10 191. The amino acid sequence according to claim 190 wherein X₁₀₅ and X₁₁₃ are cysteine residues.
192. The amino acid sequence according to claim 190 wherein X₁₀₄ and X₁₁₄ are selected from the group consisting of alanine and glycine.
193. The amino acid sequence according to claim 190 wherein X₁₀₄ is alanine and X₁₁₄ is glycine.
- 15 194. The amino acid sequence according to claim 193 wherein X₁₀₅ is valine.
195. The amino acid sequence according to claim 194 wherein X₁₁₂ is asparagine.
196. The amino acid sequence according to claim 202 wherein the affinity (K_d) of binding to IR is at least about 10⁻⁵ M.
- 20 197. An amino acid sequence which binds IR from mammalian cells comprising an amino acid sequence selected from the group listed in Figure 8.

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179. The method according to claim 178 wherein X_{105} and X_{113} are cysteine residues.
180. The method according to claim 178 wherein X_{104} and X_{114} are selected from the group consisting of alanine and glycine.
- 5 181. The method according to claim 180 wherein X_{104} is alanine and X_{114} is glycine.
182. The method according to claim 181 wherein X_{105} is valine.
183. The method according to claim 182 wherein X_{112} is asparagine.
184. The method according to claim 198 wherein the affinity (K_d) of binding to
10 IR is at least about 10^{-5} M.
185. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence selected from the group listed in Figure 8.
186. The method according to claim 185 wherein the sequence comprises
15 ACVWPTYWNCG.
187. An amino acid sequence which binds and IR and comprising an amino acid sequence of $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} are tryptophan; wherein X_{104} and X_{114} are both small amino acids; wherein X_{105} is any amino acid; and
20 wherein at least one of X_{104} , X_{105} , X_{106} and one of X_{112} X_{113} X_{114} are cysteine residues.

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172. The amino acid sequence according to claim 167 wherein X₉₂ is phenylalanine.
173. The amino acid sequence according to claim 169 wherein the amino acid sequence is HPPLSX₈₆ LX₈₈ X₈₉ X₉₀ LL.
- 5 174. The amino acid sequence according to claim 167 wherein the amino acid sequence is selected from the group consisting of HPPLEHLKAFLI, HPPLSELKLFLI, HPSLSDMRWILL, HPTSKEIYAKLL, HPTSKEIYAKLL, HPSTNQMLMKLF and HAPLSVLQALL.
- 10 175. A method modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence of X₁₀₄X₁₀₅X₁₀₆X₁₀₇X₁₀₈X₁₀₉X₁₁₀X₁₁₁X₁₁₂X₁₁₃X₁₁₄ wherein at least one of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan; wherein X₁₀₄ and X₁₁₄ are both small amino acids; wherein X₁₀₅ is any amino acid; and wherein at least one of X₁₀₄, X₁₀₅, X₁₀₆ and one of X₁₁₂ X₁₁₃ X₁₁₄ are cysteine residues.
- 15
176. The method according to claim 175 wherein at least two of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan which are separated from each other by at least two amino acids.
177. The method according to claim 176 wherein the separating amino acids are selected from the group consisting of proline, threonine and tyrosine.
- 20
178. The method according to claim 177 wherein the amino acid sequence comprises WPTYW.

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165. The method according to claim 158 wherein the amino acid sequence is selected from the group consisting of HPPLEHLKAFLI, HPPLSELKLFLI, HPSLSDMRWILL, HPTSKEIYAKLL, HPTSKEIYAKLL, HPSTNQMLMKLF and HAPLSVLQALL.
- 5 166. An amino acid sequence which binds IR, said amino acid sequence comprising $HX_{82}X_{83}X_{84}X_{85}X_{86}X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}$ herein X_{82} is proline or alanine; X_{83} is a small amino acid; X_{84} is selected from the group consisting of leucine, serine and threonine; X_{85} is a polar amino acid; X_{86} is any amino acid; X_{87} is an aliphatic amino acid; X_{88} , X_{89} , X_{90} is any amino acid; and X_{91} and X_{92} are aliphatic amino acids.
- 10
167. The amino acid sequence according to claim 166 wherein X_{82} is proline; X_{83} is selected from the group consisting of proline, serine and threonine; X_{84} is leucine; X_{85} is selected from the group consisting of glutamic acid, serine, lysine and asparagine; X_{86} is a polar amino acid; X_{87} is selected from the group consisting of leucine, methionine and isoleucine; and X_{91} and X_{92} are leucines.
- 15
168. The amino acid sequence according to claim 167 wherein X_{83} is proline.
169. The amino acid sequence according to claim 167 wherein X_{85} is serine.
170. The amino acid sequence according to claim 167 wherein X_{86} is selected from the group consisting of histidine, glutamic acid, aspartic acid and glutamine.
- 20
171. The amino acid sequence according to claim 167 wherein X_{87} is leucine.

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157. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises $HX_{82}X_{83}X_{84}X_{85}X_{86}X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}$ herein X_{82} is proline or alanine; X_{83} is a small amino acid; X_{84} is selected from the group consisting of leucine, serine and threonine; X_{85} is a polar amino acid; X_{86} is any amino acid; X_{87} is an aliphatic amino acid; X_{88} , X_{89} , X_{90} is any amino acid; and X_{91} and X_{92} are aliphatic amino acids.
158. The method according to claim 157 wherein X_{82} is proline; X_{83} is selected from the group consisting of proline, serine and threonine; X_{84} is leucine; X_{85} is selected from the group consisting of glutamic acid, serine, lysine and asparagine; X_{86} is a polar amino acid; X_{87} is selected from the group consisting of leucine, methionine and isoleucine; and X_{91} and X_{92} are leucines.
159. The method according to claim 158 wherein X_{83} is proline.
160. The method according to claim 158 wherein X_{85} is serine.
161. The method according to claim 158 wherein X_{86} is selected from the group consisting of histidine, glutamic acid, aspartic acid and glutamine.
162. The method according to claim 158 wherein X_{87} is leucine.
163. The method according to claim 158 wherein X_{92} is phenylalanine.
164. The method according to claim 160 wherein the amino acid sequence is $HPPLSX_{86}LX_{88}X_{89}X_{90}LL$.

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148. The amino acid sequence according to claim 147 wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
149. The amino acid sequence according to claim 148 wherein X_{66} is glutamic acid.
150. The amino acid sequence according to claim 149 wherein X_{63} is leucine.
151. The amino acid sequence according to claim 148 wherein X_{74} is valine.
152. The amino acid sequence according to claim 149 wherein X_{64} is glutamic acid.
153. The amino acid sequence according to claim 148 wherein X_{75} is a tyrosine.
154. The amino acid sequence accord to claim 148 wherein the amino acid sequence comprises WLDQEWAVVQCEVYGRGCPS.
155. The amino acid sequence according to claim 148 wherein the affinity (K_d) of binding to IR is at least 10^{-5} M.
156. The amino acid sequence according to claim 148 wherein the amino acid sequence comprises a sequence selected from the sequences of Figures 6A-6F.

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140. The method according to claim 139 wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
141. The method according to claim 140 wherein X_{66} is glutamic acid.
142. The method according to claim 141 wherein X_{63} is leucine.
143. The method according to claim 140 wherein X_{74} is valine.
144. The method according to claim 141 wherein X_{64} is a glutamic acid.
145. The method according to claim 141 wherein X_{75} is a tyrosine.
146. The method accord to claim 140 wherein the amino acid sequence comprises WLDQEWA~~W~~VQCEVYGRGCP~~S~~.
147. An amino acid sequence which binds IR, said amino acid sequence comprising X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81} wherein X_{62} , X_{65} , X_{66} X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.

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134. An amino acid sequence which binds IR, said amino acid sequence comprising X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆ X₅₇ X₅₈ X₅₉ X₆₀ X₆₁ wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₆₀ and X₆₁ are any amino acid; X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids; X₄₇ and X₅₉ are cysteines; X₄₈ is a polar amino acid; and X₅₁, X₅₂ and X₅₇ are small amino acids.
135. The amino acid sequence according to claim 134 wherein X₄₃ and X₄₆ are leucine; X₄₈ is selected from the group consisting of aspartic acid and glutamic acid; X₅₀ is phenylalanine or tyrosine; and X₅₁, X₅₂ and X₅₇ are glycine.
136. The amino acid sequence according to claim 135 wherein X₄₈ is glutamic acid and X₅₀ is phenylalanine.
137. The amino acid sequence according to claim 136 wherein the amino acid sequence comprises X₄₃ X₄₄ X₄₅ LCE X₄₉ FG X₅₃ X₅₄ X₅₅ X₅₆ G X₅₈ C X₆₀ X₆₁.
138. The amino acid sequence according to claim 137 wherein an amino acid sequence comprises DLRVLCELFGGAYVLGYCSE or DLRVLCELFGGAYVRGYCSE
139. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂ X₇₃ X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ X₇₉ X₈₀ X₈₁ wherein X₆₂, X₆₅, X₆₆, X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀ and X₈₁ are any amino acid; X₆₃, X₇₀, and X₇₄ are hydrophobic amino acids; X₆₄ is a polar amino acid; X₆₇ and X₇₅ are aromatic amino acids; and X₇₂ and X₇₉ are cysteines.

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127. The amino acid sequence according to claim 122 wherein the amino acid sequence is HLCVLEELFWGASLFGYCSG.
128. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the sequence $X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} X_{56} X_{57} X_{58} X_{59} X_{60} X_{61}$ wherein X_{42} , X_{43} , X_{44} , X_{45} , X_{53} , X_{55} , X_{56} , X_{58} , X_{60} and X_{61} are any amino acid; X_{43} , X_{46} , X_{49} , X_{50} and X_{54} are hydrophobic amino acids; X_{47} and X_{59} are cysteines; X_{48} is a polar amino acid; X_{51} , X_{52} and X_{57} are small amino acids.
129. The method according to claim 128 wherein X_{43} and X_{46} are leucine; X_{48} is selected from the group consisting of aspartic acid and glutamic acid; X_{50} is phenylalanine or tyrosine; and X_{51} , X_{52} and X_{57} are glycine.
130. The method according to claim 129 wherein X_{48} is glutamic acid and X_{50} is a phenylalanine.
131. The method according to claim 130 wherein the amino acid sequence is $X_{42} X_{43} X_{44} X_{45} \text{LCE} X_{49} \text{FGG} X_{53} X_{54} X_{55} X_{56} \text{GX}_{58} \text{C} X_{60} X_{61}$.
132. The method according the claim 131 wherein the amino acid sequence comprises DLRVLCELFGGAYVLGYCSE or DLRVLCELFGGAYVRGYCSE.
133. The method according to claim 128 wherein the binding to IR occurs at an affinity (K_d) of at least about 10^{-5} M.

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120. The method according to claim 116 wherein X_{23} is leucine, X_{27} is glutamic acid, X_{31} is tryptophan, and X_{32} is glycine.
121. The method according to claim 116 wherein the amino acid sequence is HLCVLEELFWGASLFGYCSG.
- 5 122. An amino acid sequence which binds IR, said amino acid sequence comprising
 $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$
wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are
any amino acid, X_{23} is any hydrophobic amino acid, X_{27} is a polar amino
10 acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid, and wherein
at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or
 X_{40} .
123. The amino acid sequence according to claim 122 wherein X_{24} and X_{39} are
cysteines.
- 15 124. The amino acid sequence according to claim 123 wherein X_{23} is selected
from methionine, valine, and leucine; X_{27} is selected from glutamic acid,
alanine, glycine, glutamine, aspartic acid and valine; X_{31} and X_{32} are small
amino acids; and X_{36} is an aromatic amino acid.
125. The amino acid sequence according to claim 122 wherein the binding to IR
20 occurs at an affinity (K_d) of at least about 10^{-5} M.
126. The amino acid sequence according to claim 124 wherein X_{23} is leucine, X_{27}
is glutamic acid, X_{31} is tryptophan, and X_{32} is glycine.

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114. An amino acid sequence which binds Site 1 of IGF-1R from mammalian cells such that binding to IR is at or below background, said sequence comprising $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
115. The amino acid sequence according to claim 114 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{18} is an aromatic amino acid; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.
116. A method of binding to Site 2 of IR from mammalian cells, said method comprising contacting said cells with an amino acid sequence comprising $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are any amino acid; X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid; and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40} .
117. The method according to claim 116 wherein X_{24} and X_{39} are cysteines.
118. The method according to claim 117 wherein X_{23} is selected from leucine, isoleucine, methionine and valine; X_{27} is selected from glutamic acid, aspartic acid, asparagine, and glutamine; X_{31} is tryptophan, X_{32} is glycine; and X_{36} is any aromatic amino acid.
119. The method according to claim 118 wherein the binding to IR occurs at an affinity (K_d) of at least about 10^{-5} M.

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106. The amino acid sequence according to claim 102 wherein amino acid X₁₈ is tryptophan.
107. The amino acid sequence according to claim 103 wherein X₂₀ is tyrosine.
108. The amino acid sequence according to claim 107 wherein X₂₁ is
5 phenylalanine.
109. The amino acid sequence according to claim 103 wherein X₁₅ is a large amino acid.
110. The amino acid sequence according to claim 101 wherein at least one amino acid is a D-amino acid.
- 10 111. The amino acid sequence according to claim 65 wherein at least one amino acid is a D-amino acid.
112. The amino acid sequence according to claim 102 wherein said amino acid sequence further comprises an amino acid extension comprising
15 X₁₀₁X₁₀₂X₁₀₃ wherein X₁₀₃ is bound to X₁₄ at the amino terminus and X₁₀₁ and X₁₀₂ are polar amino acids and X₁₀₃ is a hydrophobic amino acid.
113. The amino acid sequence according to claim 112 wherein X₁₀₁ and X₁₀₂ are independently aspartic acid or glutamic acid and X₁₀₃ is leucine, isoleucine or valine.

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98. A method of binding to Site 1 of IGF-1R from mammalian cells, said method comprising contacting IGF-1R with an amino acid sequence which binds IR and comprises the sequence of $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
99. The method according to claim 98 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{18} is an aromatic amino acid; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.
100. The method according to claim 98 wherein the amino acid sequence comprises a sequence selected from the sequences in Figures 3A through 3D.
101. An amino acid sequence which binds Site 1 of IR from mammalian cells, said sequence comprising $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
102. The amino acid sequence according to claim 101 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{20} is selected from group consisting of phenylalanine and tyrosine.
103. The amino acid sequence according to claim 102 wherein X_{14} and X_{17} are leucine.
104. The amino acid sequence according to claim 102 wherein X_{14} is leucine.
105. The amino acid sequence according to claim 102 wherein X_{17} is leucine.

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88. A method of binding to Site 1 of IR from mammalian cells, said method comprising contacting IR with an amino acid sequence which binds IR and comprises the sequence of $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
89. The method according to claim 88 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{20} is selected from group consisting of tyrosine and histidine; and X_{21} is selected from group consisting of phenylalanine and tyrosine.
90. The method according to claim 89 wherein X_{14} and X_{17} are leucine.
91. The method according to claim 89 wherein X_{14} is leucine.
92. The method according to claim 89 wherein X_{17} is leucine.
93. The method according to claim 89 wherein X_{20} is tyrosine.
94. The method according to claim 89 wherein X_{21} is phenylalanine.
95. The method according to claim 90 wherein X_{15} is a large amino acid.
96. The method according to claim 89 wherein said amino acid sequence further comprises an amino acid extension comprising $X_{101}X_{102}X_{103}$ wherein X_{103} is bound to X_{14} at the amino terminus and X_{101} and X_{102} are polar amino acids and X_{103} is a hydrophobic amino acid.
97. The method according to claim 96 wherein X_{101} and X_{102} are independently aspartic acid or glutamic acid and X_{103} is leucine, isoleucine or valine.

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85. The amino acid sequence according to claim 65 wherein the sequence comprises the amino acid sequence
 $X_{115}X_{116}X_{117}X_{118}FYX_8YFX_{11}X_{12}LX_{119}X_{120}X_{121}X_{122}$ wherein X_{115} is selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid and arginine, X_{116} is selected from the group consisting of aspartic acid, histidine, glycine and asparagine, X_{117} and X_{118} are selected from the group consisting of glycine, aspartic acid, glutamic acid, asparagine, and alanine, X_8 is selected from the group consisting of arginine, glycine, glutamic acid and serine, X_{11} is selected from the group consisting of glutamic acid, asparagine, glutamine and tryptophan, X_{12} is selected from the group consisting of aspartic acid, glutamic acid, glycine, lysine, and glutamine, X_{119} is selected from the group consisting of glutamic acid, glycine, glutamine, aspartic acid and alanine, X_{120} is selected from the group consisting of glutamic acid, aspartic acid, glycine and glutamine, X_{121} is selected from the group consisting of tryptophan, tyrosine, glutamic acid, phenylalanine, histidine and aspartic acid, and X_{122} is selected from the group consisting of glutamic acid, aspartic acid, and glycine.
86. The amino acid sequence according to claim 85 wherein X_{115} is tryptophan, X_{117} is selected from glycine, aspartic acid, glutamic acid and asparagine; X_{118} is selected from glycine, aspartic acid, glutamic acid and alanine; X_{11} , X_{119} , X_{120} , and X_{122} are glutamic acid; X_{12} is aspartic acid, and X_{121} is tryptophan or tyrosine.
87. An amino acid sequence comprising $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids and wherein said amino acid sequence binds to IR such that binding to IGF-IR is at or below background.

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79. The amino acid sequence according to claim 78 wherein X_{100} is an aliphatic amino acid.
80. The amino acid sequence according to claim 79 wherein X_{100} is leucine.
- 5 81. The amino acid sequence according to claim 68 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD or KDRIFYNGLRDLVGAVYGAWDKK.
82. The sequence according to claim 81 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD.
- 10 83. An amino acid sequence comprising an amino acid sequence selected from the group consisting of amino sequences listed in Figures 2A through 2P.
84. An amino acid sequence comprising a sequence selected from the group consisting of
- 15 SFYEAIHQLLGV,
NSFYEARMLSS,
SLNFYDALQLLA,
SSNFYQALMLLS,
SDGFYNAIELLS,
HETFYSMIRSLA,
20 HDPFYMMKSLL and
WSDFYSYFQGLD.

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70. The amino acid sequence according to claim 68 wherein X_{10} is isoleucine.
71. The amino acid sequence according to claim 68 wherein X_{10} is leucine.
72. The amino acid sequence according to claim 69 wherein X_{13} is leucine.
- 5 73. The amino acid sequence according to claim 69 wherein X_9 is tyrosine and X_{10} is phenylalanine.
74. The amino acid sequence according to claim 68 wherein the amino acid sequence comprises an amino acid sequence selected from $FYX_8X_9LX_{11}X_{12}L$, $FYX_8X_9IX_{11}X_{12}L$ and $FYX_8YFX_{11}X_{12}L$.
- 10 75. The amino acid sequence according to claim 74 wherein the amino acid sequence comprises $FYX_8X_9IX_{11}X_{12}L$.
76. The amino acid sequence according to claim 74 wherein the amino acid sequence comprises $FYX_8X_9LX_{11}X_{12}L$.
77. The amino acid sequence according to claim 74 wherein the amino acid sequence is $FYX_8YFX_{11}X_{12}L$.
- 15 78. The amino acid sequence according to claim 65 wherein the amino acid sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ further comprises amino acids X_{98} and X_{99} at the amino terminal end and X_{100} at the carboxy terminal end to form $X_{98}X_{99}X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{100}$ and wherein X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group
- 20 consisting of glycine, glutamine and proline, and X_{100} is a hydrophobic amino acid.

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62. The method according to claim 61 wherein the affinity is between about 10^{-7} M.
63. The method according to claim 48 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD or
5 KDRAFYNGLRDLVGAVYGAWD.
64. The method according to claim 48 wherein the amino acid sequence is selected from the group of amino acid sequences listed in Figures 2A through 2P.
65. An amino acid sequence comprising $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6
10 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids and wherein said amino acid sequence binds to IGF-1R.
66. The amino acid sequence according to claim 65 wherein the binding occurs at an affinity (K_d) of at least about 10^{-5} M.
- 15 67. The amino acid sequence according to claim 66 wherein the binding occurs at an affinity (K_d) of at least about 10^{-7} M.
68. The amino acid sequence according to claim 65 wherein X_6 and X_7 are phenylalanine or tyrosine, and X_{10} and X_{13} are leucine, isoleucine, tryptophan, phenylalanine or methionine.
- 20 69. The amino acid sequence according to claim 68 wherein X_6 is phenylalanine and X_7 is tyrosine.

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53. The method according to claim 50 wherein X_{13} is leucine.
54. The method according to claim 50 wherein X_9 is tyrosine and X_{10} is phenylalanine.
55. The method according to claim 50 wherein the amino acid sequence is
5 selected from $FYX_8X_9LX_{11}X_{12}L$, $FYX_8X_9IX_{11}X_{12}L$ and $FYX_8YFX_{11}X_{12}L$.
56. The method according to claim 55 wherein the amino acid sequence comprises $FYX_8X_9LX_{11}X_{12}L$.
57. The method according to claim 55 wherein the amino acid sequence comprises $FYX_8YFX_{11}X_{12}L$.
- 10 58. The method according to claim 48 wherein the amino acid sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ further comprises amino acids X_{98} and X_{99} at the amino terminal end and X_{100} at the carboxy terminal end to form $X_{98}X_{99}X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{100}$ and wherein X_{98} is optionally aspartic
15 consisting of glycine, glutamine and proline, and X_{100} is a hydrophobic amino acid.
59. The method according to claim 58 wherein X_{100} is an aliphatic amino acid.
60. The method according to claim 59 wherein X_{100} is leucine.
61. The method according to claim 48 wherein the amino acid sequence binds to
20 the insulin receptor with an affinity of at least about 10^{-5} M.

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(S295)₂₋₁₄

S-D-G-F-Y-N-A-Acy-E-L-L-S

S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib

G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib

5 N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib

GRVDWLQRNANFYDWFVAEAcyG-NH₂

and wherein underlined numbers represent a linker as defined in Table 18.

47. An amino acid sequence which specifically binds IR such that binding to IGF-1R is at or below background and wherein said amino acid sequence
10 comprises X₁X₂X₃X₄X₅ wherein X₁, X₂, and X₅ are selected from the group consisting of phenylalanine and tyrosine, X₃ is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X₄ is selected from group consisting of tryptophan, tyrosine and phenylalanine.
48. A method of modulating insulin activity in mammalian cells, said method
15 comprising administering to said cells an amino acid sequence which binds IR and comprises the sequence of amino acids X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃ wherein X₆ and X₇ are aromatic amino acids or glutamine, X₈, X₉, X₁₁ and X₁₂ may be any amino acid, X₁₀ and X₁₃ are hydrophobic amino acids.
49. The method according to claim 48 wherein X₆ and X₇ are selected from
20 group consisting of phenylalanine and tyrosine, and X₁₀ and X₁₃ are selected from group consisting of leucine, isoleucine, tryptophan, phenylalanine methionine and valine.
50. The method according to claim 48 wherein X₆ is phenylalanine and X₇ is tyrosine.
- 25 51. The method according to claim 50 wherein X₁₀ is isoleucine.
52. The method according to claim 50 wherein X₁₀ is leucine.

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14-(GRVDWLQRNANFYDWFVAE LG)
 (SDGFYNAIELLSGGG)₂₋₁₄
 H-Acy-CLEE-w-GASL-Tic-QCSG-NH₂
 RWPNFYGYFESLLTHFS-NH₂
 5 HYNAYEYFQVLLAETW-NH₂
 EGWDFYSYFSGLLASVT-NH₂
 LDRQFYRYFQDLLVGFM-NH₂
 WGRSFYRYFETLLAQGI-NH₂
 PLCFLQELFGGASLGGYCSG-NH₂
 10 WLEQERAWIWCEIQSGGCRA-NH₂
 IQGWEPFYGWFD DVVAQMFEENH₂
 TGHRLGLDEQFYWWFRDALSG-NH₂
 H-Abu-CLEE-w-GASL-Tic-QCSG-NH₂
14-(Dap-CAWPTYWNCG)₂
 15 RDHypFYDWFDDi-NH₂
 S131-14-S209
 S294-14-S210
 S295-14-S210
 S294-14-204
 20 S295-14-S204
 GFREGQRWYWFVAQVT-NH₂
 VASGHVLHGQFYRWFDQFALEE-NH₂
 VGDFCVSHDCFYGWFLRESMQ-NH₂
 DLRVLCELFGGAYVLGYCSE-NH₂
 25 HLSVGEELSWVALLGQWAR-NH₂
 APVSTEELRWGALLFGQWAG-NH₂
 ALEEEWAWVQVRSIRSGPL-NH₂
 WLEHEWAQIQCELYGRGCTY-NH₂
 AAVHEQFYDWFADQYEE-NH₂
 30 QAPSNFYDWFVREWDEE-NH₂
 QSFYDYIEELLGGEWKK-NH₂
 DPFYQGLWEWLRESGEE-NH₂
 (S204)₂₋₇
 (S204)₂₋₉
 35 (S204)₂₋₁₂
 (S204)₂₋₁₃
 DWLQRNANFYDWFVAEL-Lig
 Lig-DWLQRNANFYDWFVAEL
 (S209)₂₋₉
 40 (S210)₂₋₉
 LigKHL CVLEELFWGASLFGYCSGKKKK
 KHL CVLEELFWGASLFGYCSGKKKK-Lig
 (S294)₂₋₁₄

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SDGFYNAIELLSGGG-Lig
 KHLCVLEELFWGASLFGYCSGKK-Lig
 AFYDWFACK-Lig
 AFYEWFAKK-NH₂
 5 AFYGWFAKK-NH₂
 AFYKWFACK-NH₂
 (SDGFYNAIELLS-Lig)₂-14
 (AFYDWFACK-Lig)₂-14
 FHENAYDWFVRQVSKK
 10 FHENFADWFVRQVSKK
 FHENFYAWFVRQVSKK
 FHENFYDAFVRQVSKK
 FHENFTDWAVRQVSKK
 FQSLLEELVWGAPLFRYGTG
 15 PLCVLEELFWGASLFGQCSG
 QLEEEWAGVQCEVYGRECPs
 Cys-(Gly)₂-D117
 (Cys-(Gly)₂-D117)₂
 (S210)-14-(S212)
 20 (S131)-14-(S212)
 (S205)₂-14
 (S204)₂-14
 (S131)-14-(S210)
 RVDWLQRNANFYDWFVAELG
 25 VDWLQRNANFYDWFVAELG
 DWLQRNANFYDWFVAELG
 WLQRNANFYDWFVAELG
 LQRNANFYDWFVAELG
 QRNANFYDWFVAELG
 30 RNANFYDWFVAELG
 NANFYDWFVAELG
 ANFYDWFVAELG
 NFYDWFVAELG
 GRVDWLQRNANFYDWFVAELG-Lig
 35 Lig-GRVDWLQRNANFYDWFVAELG
 (S208)-14-(S131)
 (S208)-14-(S209)
 GRVDWLQRNANFYDWFVAEL
 GRVDWLQRNANFYDWFVAE
 40 GRVDWLQRNANFYDWFVA
 GRVDWLQRNANFYDWFV
14-(SDGFYNAIELLS-Lig)₂
 (GRVDWLQRNANFYDWFVAELG)-14

D117-b-Ala-Lys(D117)
D117-b-Ala-Dap(b-Ala-D117)
D117-Gly-Lys(Gly-D117)
D117-b-Ala-Lys(b-Ala-D117)
5 D117-Dab(D117)
D117-Orn(D117)
D117-Dap(b-Ala-D117)
D117-b-Ala-Orn(b-Ala-D117)
1-(Thia-b-Ala-D117)₂
10 FHENFYDWFVRQVS
FHENFYDWFVRQVSK
FHENFYDWFVQVSK
FHENFYDWFVVSK
FHENFYDWFVSK
15 FHENFYDWFVK
FYDWF-NH₂
FYDWFKK-NH₂
AFYDWFakk-NH₂
AAAAFYDWFAAAAKK-NH₂
20 (D117)₇₋₁₂
(Cys-Gly-D117)₂
Cys-Gly-D117
(D117)₇₋₁₄
LDALDRLMRYFEERPSL-NH₂
25 PLAELWAYFEHSEQGRSSAH-NH₂
GRVDWLQRNANFYDWFVAELG-NH₂
NGVERAGTGDNFYDWFVAQLH-NH₂
EHWNTVDPFYFTLFEWLRESG-NH₂
EHWNTVDPFYQYFSELLRESG-NH₂
30 QSDSGTVHDRFYGWFRDTWAS-NH₂
AFYDWFak-NH₂
AFYDWFA-NH₂
AFYDWF-NH₂
FYDWDA-NH₂
35 Ac-FYDWF-NH₂
Lig-FHENFYDWFVRQVSKK
Lig-GGGFHENFYDWFVRQVSKK
FHENFYDWFVRQVSKKGGG-Lig
Lig-CAWPTYWNCG
40 ACAWPTYWNCG-Lig
ACAWPTYWNCGGGG-Lig
Lig-SDGFYNAIELLS
SDGFYNAIELLS-Lig

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44. The amino acid sequence according to claim 25 wherein the sequence is selected from the group consisting of FHENFYDWFVRQVS, DYKDVTFTSAVFHENFYDWFVRQVSKK, GRVDWLQRNANFYDWFVAELG and APTFYAWFNQQT.
- 5 45. The amino acid sequence according to claim 25 wherein the sequence comprises FHENFYDWFVRQVS.
46. The amino acid sequence according to claim 25 wherein the sequence is selected from the group consisting of
- 10 FHENFYDWFVRQVAKK-NH₂
 FHENFYDWFVRQASKK-NH₂
 FHENFYDWFVRAVSKK-NH₂
 FHENFYDWFVAQVSKK-NH₂
 FHENFYDWFARQVSKK-NH₂
 FHEAFYDWFVRQVSKK-NH₂
 15 FHANFYDWFVRQVSKK-NH₂
 FAENFYDWFVRQVSKK-NH₂
 AHENFYDWFVRQVSKK-NH₂
 fhenfydwfvrqvskk
 EFHENFYDWFVRQVSEE
 20 FHENFYGWFVRQVSKK
 HETFYSMIRSLAK
 SDGFYNAIELLS
 SLNfyDALQLLAKK
 HDPFYSMMKSLK
 25 NSFYEALRMLSSK
 HPTSKEIYAKLLK
 HPSTNQMLMKLKF
 HPPLSELKLFLIKK
 HAPLSVLVQALLKK
 30 HPSLSDMRWILLK
 WSDFYSYFQGLD
 D117-Dap(D117)
 SSNFYQALMLLS
 D117-Dap(CO-CH₂-O-NH₂)
 35 HENFYGWFVRQVSKK
 D117-Lys(D117)

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36. The amino acid sequence according to claim 25 wherein the amino acid sequence $X_1X_2X_3X_4X_5$ further comprises the amino acid sequence $X_{93} X_{94} X_{95} X_{96} X_{97}$ located at the carboxy terminal end adjacent to X_5 to form $X_1X_2X_3X_4X_5X_{93}X_{94}X_{95}X_{96}X_{97}$, wherein X_{93} , X_{94} and X_{97} may be any amino acid, X_{95} is selected from the group consisting of glutamine, glutamic acid, alanine and lysine, and X_{96} is a hydrophobic or aliphatic amino acid.
37. The amino acid sequence according to claim 36 wherein X_{93} is selected from the group consisting of alanine, aspartic acid, glutamic acid, arginine, and valine, X_{95} is glutamine or glutamic acid, and X_{96} is selected from the group consisting of leucine, isoleucine, valine and tryptophan.
38. The amino acid sequence according to claim 37 wherein X_{96} is leucine or tryptophan.
39. The amino acid sequence according to claim 38 wherein X_{96} is leucine.
40. The amino acid sequence according to claim 39 wherein X_{95} is glutamine, and X_{96} is tryptophan.
41. The amino acid sequence according to claim 40 wherein X_{93} is valine.
42. The amino acid sequence according to claim 41 wherein asparagine is bound to the amino terminal of X_1 .
43. An amino acid sequence selected from the amino acid sequences listed in Figures 1-A through 1-O.

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27. The amino acid sequence according to claim 25 wherein the binding occurs at an affinity (K_d) of at least about 10^{-7} M.
28. The amino acid sequence according to claim 25 wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is
5 selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
29. The amino acid sequence according to claim 28 wherein X_3 is selected from the group consisting of aspartic acid and glutamic acid.
- 10 30. The amino acid sequence according to claim 29 wherein X_1 and X_5 are phenylalanine and X_2 is tyrosine.
31. The amino acid sequence according to claim 29 wherein X_4 is tryptophan.
32. The amino acid sequence according to claim 31 wherein X_3 is aspartic acid to result in an amino acid sequence comprising FYDWF.
- 15 33. The amino acid sequence according to claim 31 wherein X_3 is glutamic acid to result in an amino acid sequence comprising FYEWF.
34. The amino acid sequence according to claim 28 wherein the amino acid sequence FHEN is bound to the amino terminal of $X_1X_2X_3X_4X_5$ to produce an amino acid sequence comprising FHEN $X_1X_2X_3X_4X_5$.
- 20 35. The amino acid sequence according to claim 34 wherein the amino acid sequence comprises FHENFYDWF.

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- 5 DPFYQGLWEWLRESGEE-NH₂
 (S204)₂₋₇
 (S204)₂₋₉
 (S204)₂₋₁₂
 (S204)₂₋₁₃
 DWLQRNANFYDWFVAEL-Lig
 Lig-DWLQRNANFYDWFVAEL
 (S209)₂₋₉
 (S210)₂₋₉
 10 LigKHLCVLEELFWGASLFGYCSGKKKK
 KHLCVLEELFWGASLFGYCSGKKKK-Lig
 (S294)₂₋₁₄
 (S295)₂₋₁₄
 15 S-D-G-F-Y-N-A-Acy-E-L-L-S
 S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib
 G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib
 N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib
 GRVDWLQRNANFYDWFVAEAcyG-NH₂
 and wherein underlined numbers represent a linker as defined in Table 18.
- 20 22. The method according to claim 2 wherein the amino acid sequence binds to
 the insulin receptor with an affinity of at least about 10⁻⁵ M.
23. The method according to claim 22 wherein the affinity is at least about 10⁻⁷
 M.
24. The method according to claim 23 wherein the affinity is at least about 10⁻⁹
 25 M.
25. An amino acid sequence comprising X₁X₂X₃X₄X₅ wherein X₁, X₂, X₄, and
 X₅ are aromatic amino acids, X₃ is any polar amino acid, and wherein said
 amino acid sequence binds to IGF-1R.
- 30 26. The amino acid sequence according to claim 25 wherein the IGF-1R binding
 occurs with an affinity (K_d) of at least about 10⁻⁵ M.

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ANFYDWFVAELG
 NFYDWFVAELG
 GRVDWLQRNANFYDWFVAELG-Lig
 Lig-GRVDWLQRNANFYDWFVAELG
 5 (S208)-14-(S131)
 (S208)-14-(S209)
 GRVDWLQRNANFYDWFVAEL
 GRVDWLQRNANFYDWFVAE
 GRVDWLQRNANFYDWFVA
 10 GRVDWLQRNANFYDWFV
14-(SDGFYNAIELLS-Lig)₂
 (GRVDWLQRNANFYDWFVAELG)-14
14-(GRVDWLQRNANFYDWFVAE LG)
 (SDGFYNAIELLSGGG)₂-14
 15 H-Acy-CLEE-w-GASL-Tic-QCSG-NH₂
 RWPNFYGYFESLLTHFS-NH₂
 HYNIFYEYFQVLLAETW-NH₂
 EGWDFYSYFSGLLASVT-NH₂
 LDRQFYRYFQDLLVGFM-NH₂
 20 WGRSFYRYFETLLAQGI-NH₂
 PLCFLQELFGGASLGGYCSG-NH₂
 WLEQERAWIWCEIQSGGCRA-NH₂
 IQGWEPFYGWFD DVVAQMFEENH₂
 TGHRLGLDEQFYWWFRDALSG-NH₂
 25 H-Abu-CLEE-w-GASL-Tic-QCSG-NH₂
14-(Dap-CAWPTYWNCG)₂
 RDHypFYDWFDDi-NH₂
 S131-14-S209
 S294-14-S210
 30 S295-14-S210
 S294-14-204
 S295-14-S204
 GFREGQRWYWFVAQVT-NH₂
 VASGHVLHGQFYRWFVDQFALEE-NH₂
 35 VGDFCVSHDCFYGWFLRESMQ-NH₂
 DLRVLCELFGGAYVLGYCSE-NH₂
 HLSVGEELSWVALLGQWAR-NH₂
 APVSTEELRWGALLFGQWAG-NH₂
 ALEEEWAWVQVRSIRSGPL-NH₂
 40 WLEHEWAQIQCELYGRGCTY-NH₂
 AAVHEQFYDWFADQYEE-NH₂
 QAPSNFYDWFVREWDEE-NH₂
 QSFYDYIEELLGGEWKK-NH₂

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AFYDWFA-NH₂
AFYDWF-NH₂
FYDWDA-NH₂
Ac-FYDWF-NH₂
5 Lig-FHENFYDWFVRQVSKK
Lig-GGGFHENFYDWFVRQVSKK
FHENFYDWFVRQVSKKGGG-Lig
Lig-CAWPTYWNCG
ACAWPTYWNCG-Lig
10 ACAWPTYWNCGGGG-Lig
Lig-SDGFYNAIELLS
SDGFYNAIELLS-Lig
SDGFYNAIELLSGGG-Lig
KHLCVLEELFWGASLFGYCSGKK-Lig
15 AFYDWFAKK-Lig
AFYEWFAKK-NH₂
AFYGWFAKK-NH₂
AFYKWFAKK-NH₂
(SDGFYNAIELLS-Lig)₂₋₁₄
20 (AFYDWFAKK-Lig)₂₋₁₄
FHENAYDWFVRQVSKK
FHENFADWFVRQVSKK
FHENFYAWFVRQVSKK
FHENFYDAFVRQVSKK
25 FHENFTDWAVRQVSKK
FQSLLEELVWGAPLFRYGTG
PLCVLEELFWGASLFGQCSG
QLEEEWAGVQCEVYGRECPS
Cys-(Gly)₂-D117
30 (Cys-(Gly)₂-D117)₂
(S210)-14-(S212)
(S131)-14-(S212)
(S205)₂₋₁₄
(S204)₂₋₁₄
35 (S131)-14-(S210)
RVDWLQRNANFYDWFVAELG
VDWLQRNANFYDWFVAELG
DWLQRNANFYDWFVAELG
WLQRNANFYDWFVAELG
40 LQRNANFYDWFVAELG
QRNANFYDWFVAELG
RNANFYDWFVAELG
NANFYDWFVAELG

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NSFYEALRMLSSK
HPTSKEIYAKLLK
HPSTNQMLMKLFK
HPPLSELKLFLIKK
5 HAPLSVLVQALLKK
HPSLSDMRWILLK
WSDFYSYFQGLD
D117-Dap(D117)
SSNFYQALMLLS
10 D117-Dap(CO-CH₂-O-NH₂)
HENFYGWVFRQVSKK
D117-Lys(D117)
D117-b-Ala-Lys(D117)
D117-b-Ala-Dap(b-Ala-D117)
15 D117-Gly-Lys(Gly-D117)
D117-b-Ala-Lys(b-Ala-D117)
D117-Dab(D117)
D117-Orn(D117)
D117-Dap(b-Ala-D117)
20 D117-b-Ala-Orn(b-Ala-D117)
1-(Thia-b-Ala-D117)₂
FHENFYDWFVRQVS
FHENFYDWFVRQVSK
FHENFYDWFVQVSK
25 FHENFYDWFVVS
FHENFYDWFVSK
FHENFYDWFVK
FYDWF-NH₂
FYDWFKK-NH₂
30 AFYDWFakk-NH₂
AAAAFYDWFAAAAKK-NH₂
(D117)₂-12
(Cys-Gly-D117)₂
Cys-Gly-D117
35 (D117)₂-14
LDALDRLMRYFEERPSL-NH₂
PLAELWAYFEHSEQGRSSAH-NH₂
GRVDWLQRNANFYDWFVAELG-NH₂
NGVERAGTGDNFYDWFVAQLH-NH₂
40 EHWNTVDPFYFTLFEWLRESG-NH₂
EHWNTVDPFYQYFSELLRESG-NH₂
QSDSGTVHDFYGWFRDTWAS-NH₂
AFYDWFak-NH₂

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17. The method according to claim 13 wherein X_{95} is glutamic acid and the amino acid sequence is an insulin agonist.
18. The method according to claim 13 wherein asparagine is present as the amino acid bound to the amino terminal of X_1 and wherein $X_1X_2X_3X_4X_5X_{93}$ is FYDWFV
19. The method according to claim 1 wherein the amino acid sequence is selected from the group of amino acid sequences listed in Figures 1, 2, and 9.
20. The method according to claim 1 wherein the sequence is selected from the group consisting of FHENFYDWFVRQVSK,
DYKDVTFTSAVFHENFYDWFVRQVSKK,
GRVDWLQRNANFYDWFVAELG and APTFYAWFNQQT.
21. The method according to claim 1 wherein the sequence is selected from the group consisting of
- FHENFYDWFVRQVAKK-NH₂
FHENFYDWFVRQASKK-NH₂
FHENFYDWFVRAVSKK-NH₂
FHENFYDWFVAQVSKK-NH₂
FHENFYDWFARQVSKK-NH₂
FHEAFYDWFVRQVSKK-NH₂
FHANFYDWFVRQVSKK-NH₂
FAENFYDWFVRQVSKK-NH₂
AHENFYDWFVRQVSKK-NH₂
fhenfydwfvrqvskk
EFHENFYDWFVRQVSEE
FHENFYGWFVRQVSKK
HETFYSMIRSLAK
SDGFYNAIELLS
SLNFYDALQLLAKK
HDPFYSMMSLLK

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9. The method according to claim 7 wherein X_3 is glutamic acid to result in an amino acid sequence comprising FYEWF.
10. The method according to claim 1 wherein the amino acid sequence FHEN is bound to the amino terminal of $X_1X_2X_3X_4X_5$ to produce an amino acid
5 sequence comprising $FHENX_1X_2X_3X_4X_5$ and possessing insulin agonist activity.
11. The method according to claim 10 wherein the amino acid sequence is FHENFYDWF.
12. The method according to claim 1 wherein the amino acid sequence
10 $X_1X_2X_3X_4X_5$ further comprises the amino acid sequence $X_{93} X_{94} X_{95} X_{96} X_{97}$ located at the carboxy terminal end adjacent to X_5 , wherein X_{93} , X_{94} and X_{97} may be any amino acid, X_{95} is selected from the group consisting of glutamine, glutamic acid, alanine and lysine, and X_{96} is a hydrophobic or aliphatic amino acid.
13. The method according to claim 12 wherein X_{93} is selected from the group
15 consisting of alanine, aspartic acid, glutamic acid, arginine, and valine, X_{95} is glutamine or glutamic acid, and X_{96} is selected from the group consisting of leucine, isoleucine, valine and tryptophan.
14. The method according to claim 13 wherein X_{96} is leucine or tryptophan.
15. The method according to claim 14 wherein X_{96} is leucine.
20
16. The method according to claim 13 wherein X_{95} is glutamine or glutamic acid, and X_{96} is tryptophan.

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We claim:

1. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the amino acid sequence $X_1X_2X_3X_4X_5$, wherein X_1 , X_2 ,
5 X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid.
2. The method according to claim 1 wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
- 10 3. The method according to claim 2 wherein said amino acid sequence is an insulin agonist.
4. The method according to claim 2 wherein said amino acid sequence is an insulin antagonist.
5. The method according to claim either one of claims 3 or 4 wherein X_1 and
15 X_5 are phenylalanine and X_2 is tyrosine.
6. The method according to claim 5 wherein X_4 is tryptophan.
7. The method according to claim 6 wherein the amino acid sequence is an insulin agonist and X_3 is selected from the group consisting of aspartic acid and glutamic acid.
- 20 8. The method according to claim 7 wherein X_3 is aspartic acid to result in an amino acid sequence comprising FYDWF.

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3. Results

Figure 14 shows the results of one of the experiments. Clearly, IGF-1 and D8 (B12) cause a much slower dissociation rate than the 20E2 (motif 2), H2C (motif 1), C1 (motif 1), and RP6 (motif 2) peptides. This suggests that
5 IGF-1 and D8 (B12) contact IGF-1R in different locations than that of 20E2, H2C, C1, and RP6.

Previous data (EXAMPLE 28) suggests that the motif 6 series binds to a location of IGF-1R that differs from motifs 1 and 2 and that these two sites are not independent of one another. The slowing of the dissociation
10 rate by IGF-1 and D8 (B12) further suggests that there are at least two sites of binding to IGF-1R and that these two sites are not independent of one another.

The following publications, some of which have been cited herein, are
15 cited for general background information and are incorporated by reference in their entirety.

IGF-1R was sent to Wallac and the IGF-1R was labeled with Wallac's W-1024 Eu-chelate.

The Streptavidin-Allophycocyanin (SA-APC) obtained from Prozyme Cat. # PJ25S. The biotinylated 20E2

- 5 [DYKDFYDAIDQLVRGSARAGGTRDKK(ϵ -biotin)] was synthesized by Novo Nordisk or by PeptidoGenic Research & Co., Inc. The IGF-1 was commercially available from PeproTech Cat. # 100-11.

2. Methods

- 10 a. Preparation of the Assay Mix. A 1.25X concentration of Assay Mix consisting of 2.5 nM Eu-labeled IGF-1R, 18.75 nM b-20E2, 2.5 nM SA-APC, and 0.1% BSA was prepared. This mixture was allowed to pre-incubate.

b. 20 μ l of Competitor and Buffer were added to a 96-well microtiter plate (Costar Cat. #3912).

- 15 c. Wallac Victor II Fluorometer was readied to read at 665 nm only in multiple repeats (99) of only the wells containing material.

d. 80 μ l of the 1.25X Assay Mix was added to the 96-well microtiter plate and promptly placed onto the Victor II for readings.

- 20 e. After the original 99 repeat readings were taken, periodic readings were taken until equilibrium had been established.

NOTE: Different conditions can be used for these experiments. For example, a 1.1X concentration of assay mix can be initially made. Then first add 10 μ l of Competitor and Buffer to the microtiter plate followed by 90 μ l of the Assay Mix.

- 25 f. The working concentrations of this assay were 2 nM Eu-labeled IGF-1R, 15 nM b-20E2, 2 nM SA-APC, and 0.1% BSA. Peptides were normally competed at 100 μ M, whereas IGF-1 was competed at 30 μ M. Results are shown in Figure 14.

S173	rB6-4-A12-IR	5620 nM	
RP5	20E2B-3-B3-IR	7450 nM	-
G9	20E2B-1-A6-IGFR	7550 nM	-
RP4	20E2A-4-F9-IR	8110 nM	+
D8 (B12)	D820-4-B12-IR	11300 nM	
RP24	R20b-4-A4-IR	17800 nM	
RP11	A6S Design	18800 nM	+
D8	R20b-4-D8-IR	21650 nM	
A6	R40-3-A6-IGFR	46600 nM	
RP17	R20b-4-A6-IR	50000 nM	
S167	Short A6	~100 μ M	
RP3	20E2A-3-B11-IR	~100 μ M	-
KC F9	D820-4-F9-IR	~100 μ M	
JB3	CONTROL	~100 μ M	
KC G1	D820-4-F10-IGFR	~100 μ M	
C3-MDM2	CONTROL	>100 μ M	
RP21	40F-4-C1-IGFR	>100 μ M	
RP22	40F-4-D10-IGFR	>100 μ M	
RP23	40F-4-C1-IR	>100 μ M	
KC G2	D820-4-F10-IGFR	>100 μ M	
KC G7	F815-4-G7-IGFR	>100 μ M	

B. IGF-1R Peptide Assay Competition Dissociation

A competition dissociation experiment was performed to determine if any peptides altered the dissociation rate of the 20E2 (B6 motif) peptide in the IGF-1R Peptide Assay. An alteration of the dissociation rate suggests the peptide used in the competition binds to a second site on IGF-1R thus enhancing or slowing the 20E2 dissociation rate through an allosteric interaction.

1. Materials

IGF-1R was purchased from R&D System, Cat. # 391-GR/CF. The IGF-1R was labeled with Europium (Eu) by EG&G Wallac. Ten milligrams of

Compounds begin at 200 μ M in a working concentration of 2% DMSO.
Controls also contained 2% DMSO.

3. Results

The IC₅₀ and holoenzyme phosphorylation activity (see Example 20)

5 values for certain peptides are shown below.

Peptide Data

<u>Name</u>	<u>Sequence</u>	<u>IGF-1R IC₅₀</u>	<u>Holo. Phos.</u>
IGF-1	Natural Ligand	~1-10 nM	
C1	A6S-4-C1-IGFR or D112	~10 nM	
RP9	H2C Design	33 nM	++
20E2	R20a-3-20E2-IR or D118	~100 nM	
G8	20E2B-3-C6-IGFR	139 nM	-
RP2	H2CB-3-B9-IR	163 nM	+
E8	R20b-4-E8-IR or D120	175 nM	
G33	H2CA-4-G9-IGFR	178 nM	+++
RP6	20C-4-G3-IGFR	184 nM	+++++
RP14	H2CA-4-H8-IGFR	225 nM	
S178	B6C-3-C10-IR	240 nM	
RP10	20E2 Design	315 nM	+
S176	A6S-4-G1-IR	418 nM	
H2C	A6S-4-H2-IGFR or D117	~600 nM	+
B6	R40-3-B6-IGFR	631 nM	
RP13	H2CA-4-H6-IGFR	818 nM	
G8	20E2B-3-C6-IGFR	1330 nM	-
S174	R20-4-F9-IGFR	1460 nM	
RP8	20E2 Design	1800 nM	+
S177	B6C-3-C7-IR	2040 nM	
S175	A6S-3-E12-IR	2050 nM	++
RP1	H2CB-4-G11-IR	2790 nM	+
bS175	A6S-3-E12-IR	3230 nM	
NG C2	20E2-3-C2-IGFR	4020 nM	
S179	H2CBa-3-B12-IR	5350 nM	

- b. Dilutions of the Competitors were carried out on a 96-well microtiter plate (Costar Cat. #3912). 100 μ l of Buffer (TBS pH 8.0 + 0.1 % BSA) were dispensed to wells in columns 1 through 11. Competitors and Buffer were added to Column 12 wells so that the total volume is 150 μ l.
- 5 c. To identify small organic compounds which also bind the active sites of IGF-1R, dilutions of the small organic compounds are also performed on a 96-well microtiter plate (Costar Cat. #3912). Compounds are dissolved in 100% DMSO. Therefore, 100 μ l of Buffer (TBS pH 8.0 + 0.1 % BSA) with 4% DMSO are dispensed to wells in columns 1 through 10.
- 10 Column 11 contains 100 μ l of Buffer with 2.7% DMSO. Compounds (6 μ l) are added into 144 μ l Buffer (No DMSO) to Column 12 wells.
- d. Dilutions were performed across columns on the plate. Once competitors were dispensed into Column 12 and mixed, 50 μ l of the solution Column 12 were transferred to wells in 11 and mixed. 50 μ l of the Column
- 15 11 mixture was transferred to Column 10 wells. This was repeated until 50 μ l of Column 3 mixture was transferred to Column 2 wells. Once accomplished to Column 2, 50 μ l from Column 2 were removed and discarded. Column 1 wells were reserved for No Competitor Wells. 100 μ l volume was therefore maintained across all columns.
- 20 e. 50 μ l of the Assay Mix was dispensed into wells on a new 96-well microtiter plate. 50 μ l from the Dilutions Plate were then added to this plate.
- f. 30 μ l from Assay Mix Plate were transferred from the 96-well in duplicate on a 384-well microtiter plate (Nunc Cat. # 264512). This covered
- 25 plate was allowed to incubate at RT overnight.
- g. Binding was measured using Wallac's Victor II fluorometer by excitation at 340 nm and measuring emission at 665 and 615 nm.
- h. The working concentrations of this assay were 2 nM Eu-labeled IGF-1R, 15 nM b-20E2, 2 nM SA-APC, and 0.1% BSA. Peptides
- 30 were normally diluted starting from 100 μ M, where IGF-1 begins at 30 μ M.

Exempl 29: IGF-1R Peptide Assays**A. IC₅₀ Determinations**

Peptides that meet the proper criteria of affinity, selectivity, and activity may be used to develop site-directed assays to identify active molecules which bind to sites on IGF-1R. Assays have been developed using Time-Resolved Fluorescence Resonance Energy Transfer (FRET). These assays are not radioactive, homogeneous (no wash steps), and can be rapidly carried out in 96- or 384-well microtiter plate format facilitating their use in high-throughput screening assays for small organic molecules.

This assay can be used to assess the affinity of peptides for IGF-1R or can be used to find small organic molecule leads in a high-throughput capacity. The determination of the IC₅₀ for several peptides is described below.

1. Assay Components

IGF-1R was purchased from R&D System, Cat. # 391-GR/CF. The IGF-1R was labeled with Europium (Eu) by EG&G Wallac. Ten milligrams of IGF-1R was sent to Wallac and the IGF-1R was labeled with Wallac's W-1024 Eu-chelate.

The Streptavidin-Allophycocyanin (SA-APC) was obtained from Prozyme Cat. # PJ25S. The biotinylated 20E2 [DYKDFYDAIDQLVRGSARAGGTRDKK(ϵ -biotin)] ("b-20E2") was synthesized by Novo Nordisk or by PeptidoGenic Research & Co., Inc. The IGF-1 was commercially available from PeproTech Cat. # 100-11.

2. Assay Method

a. Preparation of the Assay Mix. A 2X concentration of Assay Mix consisting of 4 nM Eu-labeled IGF-1R, 30 nM b-20E2, 4 nM SA-APC, and 0.1% BSA was prepared. This mixture was allowed to pre-incubate at RT in the dark for 1-2 h before competitor was added.

times in PBST and then incubated with HRP/anti-M13 conjugate for 45 min. The plate was washed again and then the ABTS substrate added. The values indicate readings taken at OD₄₀₅. Figure 72A shows competition between cleaved monomers and dimers and F7 phage for binding to Site 2 of IR. Figure 72B shows competition for binding to Site 1 between H2C and the cleaved and uncleaved monomers and dimers. IC₅₀ values are shown in Table 20.

TABLE 20

Dimers Site 1/Site 2 IC ₅₀ Values				
Phage Signal	H2C		F8	
Cleavage	-	+	-	+
Dimers				
LF-H2C(6)F8	0.2	0.19	0.3	5
LF-H2C(9)F8	0.4	0.11	3	15
LF-H2C(12)F8	0.3	0.19	>16	16
LF-F8 mono	-	-	>20	12
LF-H2C mono	0.145	0.11	>1	>1
H2C mono	0.3	0.2	>0.5	>0.5
MBP-lacZ control	-	-	-	-

- = uncleaved

+ = cleaved

E. Stimulation of autophosphorylation of IR by MBP-Fusion Peptides

Fusion peptides were prepared as described above, and then assayed for IR activation (see Example 20). The results of these experiments shown in Figure 74 indicate that the H2C monomer and H2C-H2C homodimers stimulate autophosphorylation of IR *in vivo*.

H2C dimers (Site 1-Site 1) with a 6 amino acid linker (H2C-6-H2C) were most active in the autophosphorylation assay. Other active dimers are also shown in Figure 74, particularly H2C-9-H2C, H2C-12-H2C, H2C-3-H2C, and F8.

#428 (G3-12-G3)

AQPAMARGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRGGGTFYEW
FESALRKHGAGAAAGAPVPYPDPLEPRAALTN.

5

#434 (G3-12-G3)

ISEFIEVRAQPAMARGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRG
GGTFYEWFESALRKHGAGAAAGAPVPYPDPLEPRAA.

10

#437 (H2C)

AQPAMAFHENFYDWFVRQVSAAAGAPVPYPDPLEPRAA.

#463 (H2C-3-H2C)

15 AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAAGAPVPYPD
PLEPRAA.

#435 (H2C-3-H2C-3-H2C)

20 AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENFYD
WFVRQVSAAAGAPVPYPDPLEPRAA.

#439 (H2C-6-H2C)

25 AQPAMAFHENFYDWFVRQVSGGSGGSFHENFYDWFVRQVSAAAGAPVP
YPDPLEPRAA.

#436 (H2C-9-H2C)

AQPAMAFHENFYDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAAAG
APVPYPDPLEPRAA.

30

#449 (H2C-12-H2C)

AQPAMAFHENFYDWFVRQVSGGSGGSGGSGGSFHENFYDWFVRQVS
AAAGAPVPYPDPLEPRAA.

MBP*

35 ISEFGSSRVLDLQASLALAVVLQRRDWNPGVTQLNRLAAHPPFASWRNSEE
RTDRPSQQLRSLNGEWQLGCGFGG

40 The MBP- cleaved fusion protein mixtures were appropriately diluted,
 added to the wells, and incubated at RT for 30 min. An equal volume of F8
 or H2C phage displayed peptide was then added to each well and incubated
 for 1 h. The control wells (100% phage binding) contained only phage and
 an equal volume of buffer. The control cleaved fusion protein mixture
 contains a peptide derived from the lacZ gene. The plate was washed 3

Dimers were prepared by expressing them as MBP fusion products. See, Table 1, supra. The sequences of the MBP- cleaved dimers are shown below (core peptide sequences are underlined):

Cleaved Dimer Sequences

- 5 **#426 (D8)**
 AQPAMAWLDQEWAWWQCEVYGRGCPSAAAGAPVPYPDPLEPRAA.
- #429(D8-6-D8)**
 AQPAMAWLDQEWAWWQCEVYGRGCPSGGSGGSWLDQEWAWWQCEVY
 10 GRGCPSAAAGAPVPYPDPLEPRAA.
- #459 (short flag RB6)**
 ISEFGSADYKDLDALDRLMRYFEERPSLAAAGAPVPYPDPLEPRAA.
- 15 **#430 (H2C-4-RB6)**
 DYKDDDDKFHENFYDWFVRQVSGSGSLDALDRLMRYFEERPSLAAAGAP
 VPYPDPLEPRAA.
- #464 (H2C)**
 20 DYKDDDDDFHENFYDWFVRQVSAAAGAPVPYPDPLEPRAA.
- #446 (F8)**
 DYKDDDDHLCVLEELFWGASLFGYCSGAAAGAPVPYPDPLEPRAA.
- 25 **#431 (H2C-6-F8)**
 DYKDDDDKFHENFYDWFVRQVSGSGSGSHLCVLEELFWGASLFGYCSG
 AAAGAPVPYPDPLEPRAA.
- #433 (H2C-9-F8)**
 30 DYKDDDDKFHENFYDWFVRQVSGSGSGSGSHLCVLEELFWGASLFGY
 CSGAAAGAPVPYPDPLEPRAA.
- #432 (H2C-12-F8)**
 DYKDDDDKFHENFYDWFVRQVSGSGSGSGSGSHLCVLEELFWGASL
 35 FGYCSGAAAGAPVPYPDPLEPRAA.
- #452 (G3)**
 AQPAMARGGGTFYEWFEALRKHGAGAAAGAPVPYPDPLEPRAA.
- 40 **#427 (G3-6-G3)**
 AQPAMARGGGTFYEWFEALRKHGAGGGSGGSRGGGTFYEWFEALRK
 HGAGAAAGAPVPYPDPLEPRAA.
 (* A TO T CHANGE)

Dimers were prepared by ligation chemistry (oxime bond in the ligation site) (attached through C-terminal domain).

C. Binding of Dimers to Different IR Constructs Indicates Peptides Bind to Two Independent Sites

5 Table 19 summarizes the results of binding of phages of D117 (Formula 1 Motif), D123 (Formula 6 motif), D124 (Formula 4 motif), and CP42 (phage expressing peptide J101, Formula 8 motif) monomer to constructs of IR consisting of the L1-cys-L2 region, L1-cys-L2-FnIII α region and L2-FnIII α region.

10

TABLE 19

IR Construct	Peptides Bound	Motif
L1-cys-L2	D117	A6 Only
L1-cys-L2-FnIII α	D117, CP42, D123, 124	A6, D8, F8
L2-FnIII α	CP42, D123, D124	D8, F8

The data above is consistent with a conclusion that the A6 (Formula 1 motif) and F8 (Formula 4 Motif) motifs are physically distinct and on
15 separate parts of IR. Competition data, *supra*, further indicates that the binding site for the B6 (Formula 2 motif) is on the same subunit as that for the A6 motif.


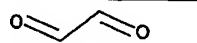
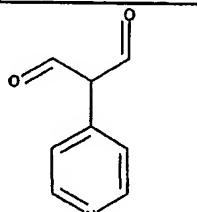
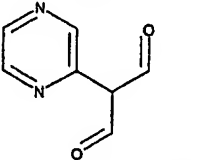
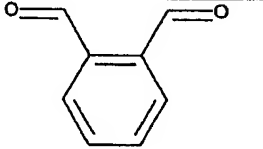
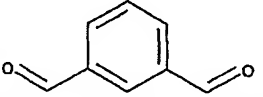
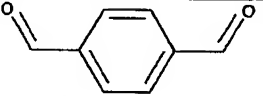
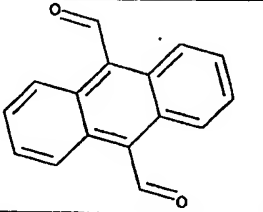
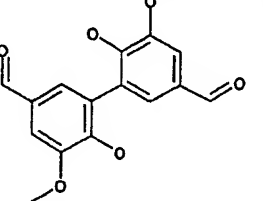

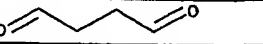
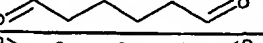


As shown below, BIAcore competition studies are consistent with the separation of Sites 1 (A6, B6) and 2 (D8, F8, J101).

20

D. Competition of Site 1 and Site 2 Phage Displayed Peptides with Recombinant Cleaved Di-Peptides

Insulin receptor was coated on a 96-well plate with 50 μ l of a 2 ng/ μ l solution of IR and incubated overnight at 4°C. The wells were then blocked with MPBS for 1 h.

TABLE 18

Structure	Number	MW	MW (- 2H ₂ O)
	<u>1</u>	100.1	64.1
	<u>2</u>	58.04	22.04
	<u>3</u>	149.15	113.15
	<u>4</u>	150.14	114.14
	<u>5</u>	134.13	98.13
	<u>6</u>	134.13	98.13
	<u>7</u>	134.13	98.13
	<u>8</u>	234.25	198.25
	<u>9</u>	302.3	266.3
	<u>10</u>	72.06	36.06
	<u>11</u>	86.09	50.09
	<u>12</u>	114.14	78.14
	<u>13</u>	128.08	92.08
	<u>14</u>	142.19	106.19

methyldimorpholine (37:2:1, v/v/v) under helium. After 2 h. at RT, the resin was washed with 5% in NMP containing 2% diethyldithiocarbamide, Na salt. Finally, the resin was washed with NMP containing HOBt (hydroxybenzotriazole). The protected oxyamino acetic acid (3 mol equiv.) was then coupled on the side-chain of Dap (diaminopropionic acid). The completion of all the acylation reactions was monitored visually by the use of bromophenol blue. Between the Fmoc-deprotection and the acylation reaction, the resin was washed with NMP (x 6).

After synthesis, the peptide was washed with DCM (dichloromethane) (x 3). The peptides were cleaved simultaneously from the resin and the side-chain protecting groups were removed by treatment with 95% aqueous TFA containing triisopropylsilan (TIS) (4 molar equiv.) for 1.5 h. The resin was rinsed with 95% aqueous acetic acid (x 4). Both TFA and acetic acid were evaporated and the peptide was finally precipitated in diethyl ether and lyophilized overnight. The peptide was both analyzed by analytical HPLC and MALDI-MS. Analysis by MALDI-MS; m/z 2287.5 (M + H)⁺ (requires m/z , 2288.3) confirmed the expected product.

To the peptide monomer, FHENFYDWFVRQVSKK-Dap(CO-CH₂-O-NH₂) (9.1 mg, 3.9 mol) was added the dialdehyde linker (0.81 mol) dissolved in 80% DMSO (aqueous) (28 l). The pH was then adjusted to 5 with solid sodium acetate. The solution was left overnight at 37°C and progress of the reaction was monitored by RP-HPLC. The formed dimer (see Table 18) was purified by semi-preparative HPLC. Analysis by MALDI-MS confirmed the expected product (see Table 18). The molecular weights and inter peptide distance of various linkers is shown below.

EXAMPLE 28: PREPARATION OF THE DIMERS**A. Materials**

Generally, suitably protected N-Fmoc (fluorenylmethoxycarbonyl)-amino acids were purchased from Novabiochem (Switzerland), 1-hydroxy-7-azabenzotriazole (HOAt) from Perspective Biosystems and *N,N'*-diisopropylcarbodiimide (DIC) from Fluka. The molecular weights of the peptides were determined using matrix-assisted laser desorption time of-flight mass spectroscopy (MALDI-MS), recorded on a Voyager-DE (Perseptive Biosystems). A matrix of sinapinic acid was used. Analytical and semi-preparative high-pressure liquid chromatography (HPLC) were performed using a Waters RCM 8 x 10 module and with a C-18 column (19 x 300 mm) and a C-18 column (25 x 300 mm), respectively, at 40°C. The solvent system for both analytical and semi-preparative HPLC was buffer A; 0.1% TFA in water and buffer B; 0.07% TFA in 100% and UV detection was at 215 nm. The gradient for analytical HPLC (1.5 ml/min); a linear gradient of 5-90% buffer B over 25 min and semi-preparative HPLC (4 ml/min); an isocratic gradient of 20% buffer B over 5 min, followed by a linear gradient of 20-60% buffer B over 40 min.

B. Solid-Phase Peptide Synthesis and Analysis of the D117 Monomer(FHENFYDWFVRQVSKK-Dap(CO-CH₂-O-NH₂))

The peptide monomer available for ligation was synthesized manually in plastic syringes using a preloaded Rink amide linker (RAM)-TentaGel (0.26 mmol/g). Fully protected N-Fmoc amino acids (3 equiv.) were used and the temporary Fmoc protecting group was removed after each cycle by 30% piperidine in *N*-methylpyrrolidone (NMP). The natural amino acids were coupled as their free acids in NMP using DIC (3 mol equiv.) and HOAt (3 mol equiv.) as coupling additive.

First, Fmoc-Dap(Alloc) was coupled as described above. The alloc group was then removed by Pd(0) (3 mol equiv.) in CHCl₃/AcOH/*N*-

TABLE 17

Phage	Sequence Formula Motif	D103 1	D118 2	D119 2	D120 10	D121 10	D122 10	D123 6	D124 4	Insulin
IM332(~J101)	Cyclic	% 85	% 100	% 100	% 100	% 100	% 100	% 100	+ 71	% 98
IM445(~J229)	APTIFYAWFNQQT	++ 0	++ 2	++ 0	++ 0	++ 0	% 100	% 100	+ 68	++ 11
IM447(~J227)	SFYEAHQHLLGV	++ 0	++ 0	++ 0	++ 0	++ 0	% 85	+ 58	+ 46	++ 3
IM452(~J228)	HPPLEHLKAFLL	++ 0	++ 0	++ 10	++ 0	++ 0	% 95	nd	% 84	+ 26
IM242(~ILPI)	ILPI	+37	++ 17	+46	+30	+66	+57	+55	++ 19	++ 0

% :>80% signal (not displaced)

+: 20-70% signal

++: <20% signal (fully displaced)

TABLE 16

IM no.	Isolate	Displayed peptide sequence	No. Found	Relative binding to			Formula
				sIR	sIGF-1R-Fc	Motif	
IM445	Δ-12-3 #6:	APTFAVWFNQQT-GGGS.	1	+++	+	1	
IM447	Δ-12-3 #45:	SFYEAHQQLLV-GGGS.	23	+++	+++	2	
IM460	Δ-12-3* #76:	NSFYEAIRMLSS-GGGS.	2	++	(+)	2	
IM453	Δ-12-3 #112:	SLNFYDALQLLA-GGGS.	1	+++	++	2	
IM466	Δ-12-3* #146	SSNFYQALMLLS-GGGS.	1	+++	++	2	
IM448	Δ-12-3 #40	SDGFYNAIELLS-GGGS.	3	+	(+)	2	
IM446	Δ-12-3 #24	HETFSMIRSLA-GGGS.	60	++++	+++	2	
IM455	Δ-12-3* #10	HDPFYSMMKSL-L-GGGS.	1	+++	++	2	
IM465	Δ-12-3 #193	WSDFYSYFQGLD-GGGS.	1	+++	(+)	2	
≥50% Consensus: _FY_AI_L_L_							
IM452	Δ-12-3 #23:	HPPLEHLKAFLL-GGGS.	4	++++	+	7	
IM451	Δ-12-3 #34:	HPPLSEKLFLI-GGGS..	33	++++	+	7	
IM459	Δ-12-3* #60:	HPSLSDMRWILL-GGGS.	2	++++	+	7	
IM458	Δ-12-3* #30:	HPAPLSVLOALL-GGGS.	2	++	+	7	
IM449	Δ-12-3 #43:	HPTSKEIYAKLL-GGGS.	14	++	+	7	
IM450	Δ-12-3 #28:	HPSTNQMLMKLF-GGGS.	40	++	+	7	
≥50% Consensus: HPPLS_L_L_L							
						S122	
						2123	

$\Delta 703$ construct and is not displaced from IR by insulin. Accordingly, J101, may bind IR outside of the insulin binding site. J101, which contains two cysteine residues is likely to have a cyclic structure.

5 Phage displaying IR binding peptides were also identified by binding phage to plates coated with sIR-Fc as discussed above and washing away non-binding phages. Binding phage were eluted with glycine-HCl, pH 2.2 for 10 min.

 The sequences of the displayed peptides which bind IR are shown in Figure 8.

10 A few of the peptides (e.g. J101 and J115) (Figure 8) were tested in the fat cell assay and all were full antagonists.

Exempl 27: Formula 8 synthetic Peptides with Their Affinities for the Human Insulin Receptor (HIR)

A commercial phage display peptide library (New England Biolabs Ph.D.-C7C Disulfide Constrained Peptide Library) was screened for
5 members which bind to IR.

A. Identification of IR Binding Phage

Binding of phage with displayed peptides was detected by ELISA assay. Plates were coated with anti-FC antibody for 2 h at RT or overnight at 4°C. Nonspecific sites were blocked with skim milk (2%) for 1 h at RT.
10 'sIR-Fc, a modified form of IR in which the cytoplasmic region is substituted with an IgG-Fc fragment (Bass *et al.*, 1990), was then added to the wells for 2 h at RT. Phage were then added to wells and incubated with or without competing peptides for 2 h at RT. Binding was detected with an anti-phage HRP antibody which was added to the wells and incubated for 2.5 h. at RT.
15 OPD (o-phenylenediamine) color reaction was detected between 5 and 10 min.

B. Characterization of Phage Displayed Peptides

Fifteen different phage were isolated from a linear 12-mer peptide library (New England Biolabs) panned against a dimer of the LI portion of IR
20 (IR Δ703) (Kristensen *et al.*, 1998) Table 16. The displayed sequences were divided into three groups based on their consensus sequences which correspond to Formula motifs 1, 2 and 7. As can be seen in Table 16, the peptides of motif 7 bind strongly to sIR but not sIGF-1R-FC.

The ability of certain peptides identified in the phage library to
25 compete with other peptides is shown in Table 17 below.

J101 (see Figure 8), the peptide expressed by phage CP42, and containing the Formula 8 motif was found to displace insulin from IR with an IC₅₀ of about 5 μm and to be an antagonist in the receptor autophosphorylation and fat cell assays. J101 also does not bind the IR

S266	H-Abu-CLEE-w-GASL-Tic-QCSG-NH ₂	$>2*10^{-5}$	0
S267	14-(Dap-CAWPTYWNCG) ₂		
S268	RDHypFYDWFDDi-NH ₂	$4.5*10^{-7}$	0
S273	S131-14-S209	$1.5*10^{-6}$	+
S274	S294-14-S210		
S275	S295-14-S210		
S276	S294-14-204		
S277	S295-14-S204		
S278	GFREGQRWYWFVAQVT-NH ₂	$>2*10^{-5}$	0
S279	VASGHVLHGQFYRWFVDQFALEE-NH ₂		
S280	VGDFCVSHDCFYGWFLRESMQ-NH ₂		
S281	DLRVLCELFGGAYVLGYCSE-NH ₂	$1.1*10^{-5}$	0
S282	HLSVGEELSWWWALLGQWAR-NH ₂	$>2*10^{-5}$	0
S283	APVSTEELRWGALLFGQWAG-NH ₂	$>2*10^{-5}$	0
S284	ALEEEWAWVQVRSIRSGPL-NH ₂	$>2*10^{-5}$	0
S285	WLEHEWAQIQCELYGRGCTY-NH ₂	$8.3*10^{-7}$	
S286	AAVHEQFYDWFADQYEE-NH ₂		
S287	QAPSNFYDWFVREWDEE-NH ₂	$5.9*10^{-6}$	0
S288	QSFYDYIEELLGGEWKK-NH ₂	$4.3*10^{-6}$	0
S289	DPFYQGLWEWLRESGEE-NH ₂	$>2*10^{-5}$	0
S290	(S204) ₂₋₇	$9.0*10^{-7}$	++
S291	(S204) ₂₋₉	$1.2*10^{-6}$	++++
S292	(S204) ₂₋₁₂	$7.5*10^{-7}$	++
S293	(S204) ₂₋₁₃	$1.2*10^{-7}$	++
S294	DWLQRNANFYDWFVAEL-Lig	$1.3*10^{-7}$	++
S295	Lig-DWLQRNANFYDWFVAEL	$4.8*10^{-7}$	+
S296	(S209) ₂₋₉		
S297	(S210) ₂₋₉		
S298	LigKHL CVLEELFWGASLFGYCSGKKKK		
S299	KHL CVLEELFWGASLFGYCSGKKKK-Lig		
S300	(S294) ₂₋₁₄	$5.0*10^{-8}$	+++
S301	(S295) ₂₋₁₄	$6.4*10^{-7}$	+
S302	S-D-G-F-Y-N-A-Acy-E-L-L-S		
S303	S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib		
S304	G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib		
S305	N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib		
S306	GRVDWLQRNANFYDWFVAEAcyG-NH ₂		

7, 9, 12, 13, and 14 represent specific chemical linkers (see Table 18)
 FFC: 0 is no effect, + is agonist, - is antagonist

S223	FHENFTDWAVRQVSKK	$>2*10^{-5}$	0
S224	FQSLLEELVWGAPLFRYGTG	$>2*10^{-5}$	0
S225	PLCVLEELFWGASLFGQCSG		
S226	QLEEEWAGVQCEVYGRECPS	$1.6*10^{-6}$	
S227	Cys-(Gly) ₂ -D117	$5.1*10^{-7}$	++
S228	(Cys-(Gly) ₂ -D117) ₂	$3.6*10^{-7}$	++
S229	(S210)-14-(S212)	$4.4*10^{-9}$	0
S230	(S131)-14-(S212)		
S231	(S205) ₂ -14	$2.7*10^{-7}$	+
S232	(S204) ₂ -14	$3.8*10^{-7}$	+++
S233	(S131)-14-(S210)	$2.6*10^{-7}$	+
S234	RVDWLQRNANFYDWFVAELG	$1.3*10^{-7}$	++
S235	VDWLQRNANFYDWFVAELG	$5.3*10^{-8}$	++
S236	DWLQRNANFYDWFVAELG	$1.0*10^{-7}$	++
S237	WLQRNANFYDWFVAELG	$8.5*10^{-7}$	0
S238	LQRNANFYDWFVAELG	$8.5*10^{-7}$	0
S239	QRNANFYDWFVAELG	$1.3*10^{-6}$	0
S240	RNANFYDWFVAELG	$1.4*10^{-6}$	
S241	NANFYDWFVAELG	$1.6*10^{-6}$	
S242	ANFYDWFVAELG	$2.0*10^{-6}$	
S243	NFYDWFVAELG	$2.0*10^{-6}$	
S244	GRVDWLQRNANFYDWFVAELG-Lig	$2.2*10^{-7}$	++
S245	Lig-GRVDWLQRNANFYDWFVAELG	$2.2*10^{-7}$	+
S246	(S208)-14-(S131)	$5.0*10^{-6}$	
S247	(S208)-14-(S209)		
S248	GRVDWLQRNANFYDWFVAEL	$6.3*10^{-8}$	++
S249	GRVDWLQRNANFYDWFVAE	$7.4*10^{-7}$	0
S250	GRVDWLQRNANFYDWFVA	$8.9*10^{-6}$	0
S251	GRVDWLQRNANFYDWFV	$5.6*10^{-6}$	
S252	14-(SDGFYNAIELLS-Lig) ₂	$4.4*10^{-7}$	0
S253	(GRVDWLQRNANFYDWFVAELG)-14	$2.2*10^{-8}$	++
S254	14-(GRVDWLQRNANFYDWFVAE LG)		
S255	(SDGFYNAIELLSGGG) ₂ -14	$1.6*10^{-6}$	0
S256	H-Acy-CLÉE-w-GASL-Tic-QCSG-NH ₂	$9.0*10^{-6}$	(-)
S257	RWPNFYGYFESLLTHFS-NH ₂	$1.4*10^{-5}$	0
S258	HYNAFYEYFQVLLAETW-NH ₂		
S259	EGWDFYSYFSGLLASVT-NH ₂	$7.7*10^{-6}$	0
S260	LDRQFYRYFQDLLVGFM-NH ₂	$2.3*10^{-6}$	0
S261	WGRSFYRYFETLLAQGI-NH ₂	$>2*10^{-5}$	0
S262	PLCFLQELFGGASLGGYCSG-NH ₂	$1.9*10^{-5}$	0
S263	WLEQERAWIWCEIQGSGCRA-NH ₂	$>2*10^{-5}$	0
S264	IQGWEPFYGWFDVVQMFEE-NH ₂	$1.9*10^{-7}$	0
S265	TGHRGLDEQFYWWFRDALSG-NH ₂	$1.1*10^{-7}$	0

S160	FHENFYDWFVSK		
S161	FHENFYDWFVSK		
S162	FHENFYDWFVK		
S165	FYDWF-NH ₂	$>2*10^{-5}$	0
S166	FYDWFKK-NH ₂	$>2*10^{-5}$	0
S167	AFYDWFKK-NH ₂	$>2*10^{-5}$	(-)
S168	AAAAFYDWFAAAAKK-NH ₂	$3.8*10^{-6}$	0
S169	(D117) ₂₋₁₂	$5.8*10^{-7}$	++
S170	(Cys-Gly-D117) ₂	$7.0*10^{-7}$	+++
S171	Cys-Gly-D117	$2.9*10^{-6}$	+++
S172	(D117) ₂₋₁₄	$4.8*10^{-6}$	+++
S173	LDALDRLMRYFEERPSL-NH ₂	$1.2*10^{-6}$	0
S174	PLAELWAYFEHSEQGRSSAH-NH ₂	$1.6*10^{-5}$	0
S175	GRVDWLQRNANFYDWFVAELG-NH ₂	$2.3*10^{-7}$	+++
S176	NGVERAGTGNFYDWFVAQLH-NH ₂	$4.7*10^{-7}$	+
S177	EHWNTVDPFYFTLFEWLRESG-NH ₂	$2.7*10^{-6}$	0
S178	EHWNTVDPFYQYFSELLRESG-NH ₂	$1.3*10^{-7}$	++
S179	QSDSGTVHDRFYGWFRDTWAS-NH ₂	$5.4*10^{-7}$	+
S180	AFYDWFKA-NH ₂	$>2*10^{-5}$	0
S181	AFYDWFA-NH ₂	$>2*10^{-5}$	0
S182	AFYDWF-NH ₂	$>2*10^{-5}$	0
S183	FYDWDA-NH ₂	$>2*10^{-5}$	0
S184	Ac-FYDWF-NH ₂	$>2*10^{-5}$	0
S203	Lig-FHENFYDWFVRQVSKK		
S204	Lig-GGGFHENFYDWFVRQVSKK		
S205	FHENFYDWFVRQVSKKGGG-Lig		
S206	Lig-CAWPTYWNCG		
S207	ACAWPTYWNCG-Lig		
S208	ACAWPTYWNCGGGG-Lig		
S209	Lig-SDGFYNAIELLS		
S210	SDGFYNAIELLS-Lig		
S211	SDGFYNAIELLSGGG-Lig		
S212	KHLCVLEELFWGASLFGYCSGKK-Lig		
S213	AFYDWFKA-Lig		
S214	AFYEWFAKK-NH ₂	$>2*10^{-5}$	0
S215	AFYGWFAKK-NH ₂	$>2*10^{-5}$	0
S216	AFYKWFAKK-NH ₂	$>2*10^{-5}$	0
S217	(SDGFYNAIELLS-Lig) ₂₋₁₄	$3.9*10^{-8}$	++
S218	(AFYDWFKA-Lig) ₂₋₁₄	$1,1*10^{-5}$	0
S219	FHENAYDWFVRQVSKK	$>2*10^{-5}$	0
S220	FHENFADWFVRQVSKK	$>2*10^{-5}$	0
S221	FHENFYAWFVRQVSKK	$1.1*10^{-6}$	(+)
S222	FHENFYDAFVRQVSKK	$>2*10^{-5}$	0

TABLE 15

Name	Sequenc	HIR affinity mol/l	FFC
S105	FHENFYDWFVRQVAKK-NH ₂	$3.1 \cdot 10^{-7}$	++
S106	FHENFYDWFVRQASKK-NH ₂	$4.2 \cdot 10^{-7}$	++
S107	FHENFYDWFVRAVSKK-NH ₂	$10.0 \cdot 10^{-7}$	+
S108	FHENFYDWFVAQVSKK-NH ₂	$7.5 \cdot 10^{-7}$	+
S109	FHENFYDWFARQVSKK-NH ₂	$2.3 \cdot 10^{-7}$	++
S110	FHEAFYDWFVRQVSKK-NH ₂	$2.2 \cdot 10^{-7}$	++
S111	FHANFYDWFVRQVSKK-NH ₂	$3.3 \cdot 10^{-7}$	0
S112	FAENFYDWFVRQVSKK-NH ₂	$6.1 \cdot 10^{-7}$	+
S113	AHENFYDWFVRQVSKK-NH ₂	$5.9 \cdot 10^{-7}$	+
S114	fhenfydwfvrqvskk	$8.3 \cdot 10^{-6}$	0
S115	EFHENFYDWFVRQVSEE	$6.5 \cdot 10^{-7}$	+
S116	FHENFYGWFVRQVSKK	$1.4 \cdot 10^{-6}$	++
S117	HETFYSMIRSLAK	$2.7 \cdot 10^{-6}$	0
S118	SDGFYNAIELLS	$2.4 \cdot 10^{-6}$	+
S119	SLNFYDALQLLAKK	$1.8 \cdot 10^{-6}$	0
S120	HDPFYSMMSLLK	$2.0 \cdot 10^{-6}$	0
S121	NSFYEALRMLSSK	$3.1 \cdot 10^{-6}$	0
S122	HPTSKEIYAKLLK	$9.3 \cdot 10^{-6}$	0
S123	HPSTNQMLMKLKF	$1.6 \cdot 10^{-5}$	0
S124	HPPLSELKLFLIKK	$2.3 \cdot 10^{-5}$	0
S125	HAPLSVLVQALLKK		0
S126	HPSLSDMRWILLK		
S127	WSDFYSYFQGLD	$1.2 \cdot 10^{-6}$	0
S128	D117-Dap(D117)	$1.1 \cdot 10^{-5}$	++
S129	SSNFYQALMLLS	$2.9 \cdot 10^{-6}$	0
S131	D117-Dap(CO-CH ₂ -O-NH ₂)	$1.2 \cdot 10^{-6}$	+
S137	HENFYGWFWVRQVSKK	$7.7 \cdot 10^{-7}$	0
S145	D117-Lys(D117)	$1.5 \cdot 10^{-6}$	++
S147	D117-b-Ala-Lys(D117)	$9.3 \cdot 10^{-7}$	++
S148	D117-b-Ala-Dap(b-Ala-D117)	$1.1 \cdot 10^{-6}$	++
S149	D117-Gly-Lys(Gly-D117)	$2.0 \cdot 10^{-6}$	++
S150	D117-b-Ala-Lys(b-Ala-D117)	$6.2 \cdot 10^{-7}$	++
S152	D117-Dab(D117)	$5.2 \cdot 10^{-6}$	+
S153	D117-Orn(D117)	$3.9 \cdot 10^{-6}$	+
S154	D117-Dap(b-Ala-D117)	$3.6 \cdot 10^{-6}$	+
S155	D117-b-Ala-Orn(b-Ala-D117)	$2.5 \cdot 10^{-6}$	++
S156	1-(Thia-b-Ala-D117) ₂		
S157	FHENFYDWFVRQVS		
S158	FHENFYDWFVRQVSK	$8.1 \cdot 10^{-7}$	+
S159	FHENFYDWFVQVSK		

Results:

The binding assays showed that most of the peptides completely inhibited insulin binding to HIR with IC_{50} -values ranging from 0.3 to 20 μ M.

- 5 One peptide (D124) was active at lower concentration but only displaced insulin partially (see Figure 71). One peptide (D112) had high affinity for HIGF-1R, but all the others showed 2-20 fold selectivity for HIR (see Figure 71).

- 10 In the effect assay (FFC), several of the peptides had no effect, some were antagonists, and a few were agonists reaching a response comparable to that of full insulin stimulation. The ED_{50} for the best peptides (D113 and D117) was around 20-30 μ M.

- 15 Despite a right shifted dose response curve relative to insulin, these peptides represent the first non-insulin compounds ever found to elicit a maximal insulin response by binding to the insulin receptor. Such peptides may be useful for development as therapeutics themselves.

The peptides could also be useful as leads for further characterization of molecular requirements for binding to and activation of IR, and/or as tools for identification of the mechanisms involved in the activation.

- 20 Analysis of affinity and activity of another group of peptides is shown in Table 15. In addition to presenting data on the single chain or looped peptide, Table 15 also reports data showing high affinity binding of certain dimers.

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TABLE 14

Comp. 1	Resp. 2	#expts 3	ED ₅₀ 4	Comments 5
D101	0	4		
D102	0	2		Precipitates
D103	0	2		
D104	0	2		Precipitates
D105	0	2		
D106	0	2		Precipitates
D107	-2	2		
D108	-1	2		
D110	-1	2		
D110	-2	4		
D111	0	2		
D112	0	5		Precipitates
D113	+2	7	Approx 20 μ M	Insoluble, especially after freeze-thaw, resulting in inconsistent results. Some response at basal insulin.
D114	0	2		
D115	0	3		
D116	+2	4	> 20 μ M	Slight effect at basal insulin
D117	+2	8	Approx 20 μ M	Precipitates. Under assay conditions, soluble at least up to 20 μ M (no ppt in microscope, low magnification). Some response at basal insulin.
D117K	+2	2	> 20 μ M	
D118	+2	5	Approx 20 μ M	Biphasic dose response curve (needs repeating)
D119	+1	2		
D120	-1	4		
D121	-1	3		
D122	-1	6		
D123	-1	5		Precipitates
D124	0	5		Precipitates
D125	0	2		
D126	0	2		

¹ Includes series "A" e.g. D101A

² Subjective ranking, on a scale of -2 (antagonist) to +2 (agonist)

³ Includes experiments run at basal and sub maximal insulin concentrations

⁴ Estimated, not calculated values.

⁵ "Precipitates" indicates precipitate in diluted stock prior
to adding to assay. May be soluble under assay conditions

**Exempl 26: Determination of Insulin Agonist Activity
Based On ^3H -Glucose Uptake into Adipocytes**

- Insulin increases uptake of ^3H glucose into adipocytes and its conversion into lipid. Incorporation of ^3H into the lipid phase was determined by partitioning of lipid phase into a scintillant mixture, which excludes water-soluble ^3H products. The effect of compounds on the incorporation of ^3H glucose at a sub-maximal insulin dose was determined, and the results expressed as increase relative to full insulin response. The method was adapted from Moody *et al.* (1974).
- 10 Mouse epididymal fat pads were dissected out, minced into degradation buffer (Krebs-Ringer 25 mM HEPES, 4% HSA, 1.1 mM glucose, 0.4 mg/ml Collagenase Type 1, pH 7.4), and degraded for up to 1.5 h at 36.5°C. After filtration, washing (Krebs-Ringer HEPES, 1% HSA) and resuspension in assay buffer (Krebs-Ringer HEPES, 1% HSA), cells were
- 15 pipetted into 96-well Picoplates (Packard), containing test solution and approximately an ED_{20} insulin. The assay was started by addition of ^3H glucose (Amersham TRK 239), in a final concentration of 0.45 mM glucose. The assay was incubated for 2 h, 36.5°C, in a Labshaker incubation tower, 400 rpm, then terminated by the addition of Permablend/Toluene scintillant
- 20 (or equivalent), and the plates sealed, before standing for at least 1 h and detection in a Packard Top Counter or equivalent. A full insulin standard curve (8 dose) was run as control on each plate. Data are presented graphically, as effect of compound on an (approx) ED_{20} insulin response, with data normalized to a full insulin response. The assay can also be run at
- 25 basal or maximal insulin concentration. Representative dose-response curves for insulin and IGF-1 are shown in figures 71A-71Z; 71A2-71Z2; 71A3-B3. Qualitative references are shown in Table 14.

TABLE 13

Name	K_d (μM) HIR	K_d (μM) HIGF1R	Ratio	Autophosph. Blot
D101	0.51	13	25	-
D102	1.2	7.4	6.2	-
D103	0.74	15	20	-
D104	20	>20		-
D105	2.8	12	4.3	-
D106	0.97	6.2	6.4	-
D107	1.1	9.7	8.8	+
D108	2.3	19	8.3	-
D109	3.6	12	3.3	-
D110	0.84	1.4	1.7	-
D111	0.62	3.2	5.2	-
D112	0.49	0.05	0.1	-
D113	0.75	5.4	7.2	- (prec)
D114	8.1	>20	>2.5	0
D115	8.1	>20	>2.5	0
D116	4.4	8.1	1.8	0
D117	0.70	6.1	8.6	+
D117K	0.82	9.1	11.1	
D118	0.25	1.3	5.2	+
D119	4.5	13	2.9	+
D120	0.37	2.2	5.9	-
D121	1.1	7.4	6.7	-
D122	1.2	>20	>17	0
D123	0.55	16	29	0
D124	0.04*	8.2	200	-
D101A	0.27	11.0	41	
D102A	0.97	16.0	16	
D112A	0.19	0.02*	0.1	
D113A				
D117A	0.60	5.1	8.5	
D119A	3.0	2.5	0.8	
D122A	1.0	>20	>20	
D123A	1.3	>20	>15	
D124A	0.09*	>20	>200	
D125A	2.6	>20	>8	
D126A	1.4	18	13	

TABLE 12

Name	Sequence	Motif
D101	KIGGQGHQDGNFYDWFVEALAKK	1
D102	KVLQARHGCDSVSDCFYEWFAKK	1
D103	KWSALLSVMDTGIFYAWFDDAVKK	1
D104	KGHSWALVRHVDRLFYEWFDLKK	1
D105	KRDKPTDQEEQNWSFYEFRHKK	1
D106	KVFWNCRSQQLDFYEWFEQAACK	1
D107	KLESHYVVPQAALDRLFYSWFSKK	1
D108	KFYGWFSRQLSLTPRDDWGLPKK	1
D109	KSAPGLVSNKQDGLFYSWFREKK	1
D110	KRGGGTIFYEFESALRKHGAGKK	1
D111	KDPERMQSDVGIFYEFRAAVGKK	1
D112	DYKDCWARPCGDAANFYDWFVQQASKK	1
D113	DYKDVTFTSAVFHENFYDWFVRQVSKK	1
D114	SAKNFYDWFVKK	1
D115	ADKNFYDWFMAAKK	1
D116	DYKDLCSWGVRIWLAGLCPKK	9
D117	FHENFYDWFVRQVSKK	1
D117K	KFHENFYDWFVRQVSKK	1
D118	DYKDFYDAIDQLVRGSARAGGTRDKK	2
D119	KDRAFYNGLRDLVGAVYGAWDKK	2
D120	KVRGFQGGTVWPGYEWLRNAACK	10
D121	KSMFVAGSDRWPGYGVLDWLKK	10
D122	KEIEAEWGRVRCLVYGRCVGGKK	10
D123	KWLDQEWAWVQCEVYGRGCPSKK	6
D124	KHLCVLEELFWGASLFGYCSGKK	4
D101A	KIGGQGHQDGNFYDWFVEALAKK	1
D102A	KVLQARHGCDSVSDCFYEWFAKK	1
D112A	DYKDCWARPCGDAANFYDWFVQQASKK	1
D113A	DYKDVTFTSAVFHENFYDWFVRQVSKK	1
D117A	FHENFYDWFVRQVSKK	1
D119A	KDRAFYNGLRDLVGAVYGAWDKK	2
D122A	KEIEAEWGRVRCLVYGRCVGGKK	10
D123A	KWLDQEWAWVQCEVYGRGCPSKK	6
D124A	KHLCVLEELFWGASLFGYCSGKK	4

for facilitate solubility. Peptides produced recombinantly by phage are indicated as D1XXA.

The peptides are all biotinylated in the side chain of the C-terminal lysine (except D117A). The peptides produced recombinantly are C-terminal acids, whereas the synthetic peptides are C-terminal amides.

5

The results of the binding and phosphorylation assays are shown in Table 13.

that C1 and H2C at 30 μ M inhibit binding to 40-50% of control and C1C at 30 μ M inhibit to 60%. B6 and growth hormone do not compete with binding of 12H10 to IR. Four MBP-peptide fusion proteins (D10, 20A4, E7 and H8) all inhibit binding of 12H10 to IR to 20-30 % of control (Figure 69).

5 **Example 24: Effects of Small Organic Molecules on IR Phosphorylation**

Organic molecules positive for binding to IGF-1R and negative controls can be tested for their effects on phosphorylation of insulin receptor.

10 **Example 25: Method for Determination of Insulin Receptor Binding of Peptides**

In other insulin binding assays, IR was incubated with 125 I-labeled insulin at various concentrations of test substance and the K_d was calculated. According to this method, human insulin receptor (HIR) or human IGF-1 receptor (HIGF-1R) was purified from transfected cells after
15 solubilization with Triton X-100. The assay buffer contains 100 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM MSG, 0.5% human serum albumin, 0.2% gammaglobulin and 0.025% Triton X-100. The receptor concentration was chosen to give 30-60% binding of 2000 cpm (3 pM) of its 125 I-labeled ligand (TyrA14- 125 I-HI or Tyr31- 125 I-IGF1) and a dilution series of the substance to
20 be tested was added. After equilibration for 2 days at 4°C, each sample (200 μ l) was precipitated by addition of 400 μ l 25% PEG 6000, centrifuged, washed with 1 ml 15% PEG 6000, and counted in a gamma-counter.

The insulin/IGF-1 competition curve was fitted to a one-site binding model and the calculated parameters for receptor concentration, insulin
25 affinity, and non-specific binding were used in calculating the binding constants of the test substances. Representative curves for insulin and IGF-1 are shown in Figures 71A-71N.

The sequences of certain peptides analyzed are shown in Table 12, except for peptides D125 and D126. Synthetic peptides are numbered
30 D1XX. D117K is an analog of D117 with an extra N-terminal lysine added

(EG&G Wallac) in Tris-buffered saline containing 0.1% BSA is added to 96- or 384-well white low-fluorescence plates (Nunc) for 2 h or overnight. For library screening, 20 μ M of small organic molecules in 2 % DMSO are included in the mixture. Unlabeled rVab 43G7 at 50 nM or IGF at 3 μ M are used as positive controls. Fluorescence of samples in each well is measured at both 615 nm and 665 nm using a VICTOR 1420 Multilabel Counter (EG &G Wallac).

Example 22: Binding of Synthetic Peptides to Insulin Receptor

A series of synthetic peptides were synthesized and biotinylated (Anaspec, Inc., San Jose, CA). The binding affinities of these peptides to IR and IGF-1R were tested. Most of these peptides bind to IR at micromolar range (Figure 63). Comparison of binding of biotinylated C1 peptide to IGF-1R and IR is shown in Figure 64, which indicates that binding of C1 to IGF-1R is at the nM range while binding to IR is at the micromolar range. A series of unlabeled peptides or soluble rVab were added to test competition binding to IR (Figure 65). H2C peptide at 30 μ M appears to compete for binding to IR with biotinylated peptides from group 1 (Formula 1 motif) (20D1 and 20D3) and the two A6-based peptides (C1 and H2) but not compete with peptides from group 2 (Formula 6 motif) (20A4 and D8), group 3 (Formula 2 motif) (20C11) or the IGF peptide A9. The 33 F7 soluble rVab antibody competes with group 1 and 2 peptides as well as C1 peptide, however, it does not compete with 20C11 or 2A9. Figure 66 shows that H2C competition with biotinylated peptides, 20D3, H2, and C1, binding to IR is dose-dependent. C1C peptide also competes with C1 for IR binding (Figure 67).

Example 23: Competition for Binding to rVab 12H10 by Peptides and MBP-Peptide Fusion Proteins

Several peptides and four MBP-peptide fusion peptides were tested for competition of binding to IR with soluble rVab 12H10. Figure 68 shows

Hercules, CA). The samples were run on 4-12% SDS-polyacrylamide gels. Western Blot analysis was performed by transferring the proteins onto nitrocellulose membrane. The membrane was blocked in PBS containing 3% milk overnight. The membrane was incubated with anti-phosphotyrosine
5 4G10 HRP labeled antibody (Upstate Biotechnology) for 2 h. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate Chemiluminescence Detection System (Pierce Chemical Co.).

**Example 21: Development of IR Assays Using Soluble
rVab Antibodies and Biotinylated Peptides**

- 10 a. Heterogeneous Time-Resolved Fluorescence Assay. Sixty microliters of insulin receptor (60 ng/well) was coated onto 96-well low-fluorescence MaxiSorp (Nunc) plates overnight at 4°C. The plates were blocked with TBS containing 2% milk and 0.5% BSA for 1 h at RT followed by three TBS washes. To test binding of peptides to insulin receptor, serial
15 dilutions of biotinylated peptides were added to IR coated plates for 2 h to overnight. After TBS wash, europium-labeled streptavidin at 1 µg/ml in assay buffer (100 mM Tris-HCl, pH 7.8; 150 mM NaCl; 0.5% BSA; 0.05% bovine Ig; 0.05% NaN₃; 0.01% Tween-20) was added to the plates and incubated for 1 h. To test binding of rVab antibodies to IR, Eu-labeled rVab
20 antibodies in assay buffer were added to the plates and incubated for 2 h to overnight. After incubation with Eu-labeled streptavidin (for peptide test) or europium-labeled rVabs, the plates were washed 5 times with Tris-buffered saline (pH 7.5) containing 0.1% Tween-20 (TTBS) and tapped dry. Sixty
25 microliters of EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen phthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG & G Wallac).
- 30 b. Homogeneous Time-Resolved Fluorescence Assay. A mixture of 27 nM Cy5-labeled rVab 43G7 and 6-8 nM LANCE-labeled IGF-1R

al., 1993; clone 969). The assay procedure begins with the cell stimulation. The IR transfected 32D cells were seeded at 5×10^6 cells/well in 96-well tissue culture plates and incubated overnight at 37°C. Samples were diluted 1:10 in the stimulation medium (PRIM1640 with 25 nM HEPES pH 7.2) plus
5 or minus insulin. The culture media was decanted from the cell culture plates, and the diluted samples were added to the cells. The plates were incubated at 37°C for 30 min. The stimulation medium was decanted from the plates, and cell lysis buffer (50 mM HEPES pH 7.2, 150 mM NaCl, 0.5% Triton X-100, 1 mM AEBSF, 10 KIU/ml aprotinin, 50 µM leupeptin, and 2 mM
10 sodium orthovanadate) was added. The cells were lysed for 30 min.

In the ELISA portion of the assay, the cell lysates were added to the BSA-blocked anti-IR unit mAb (Upstate Biotechnology, Lake Placid, NY) coated ELISA plates. After a 2 h incubation, the plates were washed 6 times with PBST and biotinylated anti-phosphotyrosine antibody (Upstate
15 Biotechnology) is added. After another 2 h incubation, the plates were again washed 6 times. Streptavidin-Eu was then added, and the plates were incubated for 1 h. After washing the plates again, EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen phthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide;
20 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG&G Wallac).

Alternatively, IR autophosphorylation was determined using a
25 holoenzyme phosphorylation assay. In accordance with this assay, 1 µl of purified insulin receptor (isolated from a Wheat Germ Agglutinin Expression System) was incubated with 25 nM insulin, or 10 or 50 µM peptide in 50 µl autophosphorylation buffer (50 mM HEPES pH. 8.0, 150 mM NaCl, 0.025% Triton-X-100, 5 mM Mn₂Cl, 50 µM sodium orthovanadate) containing 10 µM
30 ATP for 45 min at 22°C. The reaction was stopped by adding 50 µl of gel loading buffer containing β-mercaptoethanol (Bio-Rad Laboratories, Inc.,

Example 9. Prior to addition of soluble rVabs to the plates, 100 l/well of 100 nM insulin solution in PBS was added to duplicate wells. After incubation for 1 h at RT, the prepared soluble rVabs were added to each well (100 µl/well) without removing the insulin solution. After incubation for 1 h at RT, the
5 wells were washed and the color was developed as described in Example 9.

Example 19: Activities of rVabs in the Cell-Based Assay

Agonistic and antagonistic activities of IR-specific soluble rVabs were tested in 969 cells stably transfected with the gene encoding the human IR and IRS-1 (insulin receptor substrate). The resulting cell line requires IL-3,
10 IL-4, or insulin for growth. Negative control cell lines do not require IRS-1 for growth. The cells were grown in RPMI 1640 media containing 10% FCS and 20 units of IL-3 per ml. Cells were seeded at 30,000 cells/well in 50 µl PRMI1640 (without IL-3) media containing horse serum instead of FCS to reduce the background. Fifty microliters of either insulin or soluble rVabs at
15 different concentrations were added to duplicate wells, followed by incubation for 18 h in a CO₂ incubator. The cell proliferation assays were performed using WST-1 reagent. The WST-1 tetrazolium salt is cleaved to form formazan by the succinate-tetrazolium reductase system that is active only in viable cells. An increase in the number of cells results in an increase
20 of the overall enzymatic activity of the dehydrogenase that results in a higher absorbance at 450 nm. Ten microliters of WST-1 reagent were added and the plate was incubated for 1-4 h at 36°C. Figure 60 shows the results of these studies. As can be seen, rVab 12h10 was able to induce an agonist response in 32D cells expressing IR with an ED₅₀ of approximately
25 50 nM.

Example 20: IR Activation Assays

The kinase receptor activation ELISA is a functional assay based on the ability of a sample to stimulate or inhibit autophosphorylation of the insulin receptor construct that has been transfected into 32D cells (Wang et

pelleted cells were resuspended in 25 ml of 2xYT-AI media (no glucose is added) and were incubated overnight at 30°C with shaking (250 rpm).

E. Purification of rVabs

The Pharmacia RPSA Purification Module kit was used (Cat. #17-1362-01), and purification was performed according to the manufacturer's directions.

- a. A syringe was filled with the Elution Buffer (100 mM glycine, pH 3.0).
- b. The stopper on the top of the anti-E-Tag column was removed and a drop of the Elution Buffer was added to the top of the column. The syringe was connected to the column with the Luer adapter. The connection was "drop to drop" to avoid introducing air into the column.
- c. The twist-off end was removed and the column was washed with 15 ml of the Elution Buffer at a flow rate of 5 ml/min, followed immediately by 25 ml Binding Buffer (10X Binding Buffer: 0.20 M Phosphate Buffer, 0.05% NaN₃, pH 7.0).
- d. Sample was applied with a peristaltic pump P-1 (Pharmacia, Cat. #19-4611-02) at a flow rate of 5 ml/min at 4°C.
- e. The column was washed with 25 ml of the Binding Buffer at a flow rate of 5 ml/min to remove unbound *E. coli* proteins.
- f. Bound rVab was eluted from the anti-E-Tag column with the Elution Buffer. The first 4.5 ml of material eluted from the column was discarded.
- g. The next 5 ml (containing the purified E-tagged rVab) was collected in either one or several fractions.
- h. The column was immediately re-equilibrate with 25 ml of the Binding Buffer for use with the next sample.

Example 18: Competition ELISA with rVabs

For IC₅₀ determinations, microtiter plates were coated with IR and blocked as in Example 9. Soluble rVabs were prepared as described in

g. Eighty microliters of the MPBS blocking buffer was added to each tube of plate S2 (already containing 320 μ l of the supernatant) and incubated for 10 min at RT. This rVab preparation was now ready to be used in an ELISA performed described above.

5 **C. Detection of rVab Binding Using HRP/Anti-E-Tag Conjugate**

a. A microtiter plate was coated with the target protein and blocked as previously described. Some of the wells of the microtiter plate were coated with an unrelated antigen to serve as a negative control.

10 b. The rVab preparation prepared above was diluted two-fold with the MPBS blocking buffer. Two hundred microliters of this solution was added to a set of antigen-coated and control wells.

c. The plate was incubated for 2 h at RT, and then washed 3 times with PBST.

15 d. The HRP/Anti-E-Tag conjugate was diluted 1:4,000 in the MPBS blocking buffer. Two hundred microliters of the diluted conjugate was added to each well, and the plate was incubated for 1 h at RT.

e. The microtiter plate was washed 3 times with PBST.

20 f. Two hundred microliters of the ABTS solution was added to each well, the microtiter plate was incubated for 20 min at RT, and the absorbance of each well was read at 405 nm in an appropriate microtiter plate reader.

D. Production of Soluble rVabs

25 a. A suitable rVab clone in HB2151 cells was transferred from a 2xYT plate to 3 ml of 2xYT-AG media, and the culture was incubated overnight at 30°C with shaking (250 rpm).

b. Part of the overnight culture (2.5 ml) was added to 25 ml of the 2xYT media and incubated for 1 h at 30°C with shaking (250 rpm).

30 c. The culture was centrifuged at 1000 g for 20 min at RT, and the supernatant was removed from the pelleted cells and discarded. The

b. Fifty microliters of the pool phage from biopanning round 3 (or round 4) were transferred to 2 ml of the log phase HB2151 cells. The cells were incubated with gentle shaking for 1 h at 37°C.

c. The cells were diluted appropriately with the 2xYT media,
5 plated on 2xYT-AGN plates and incubated overnight at 30°C.

**B. Preparation of Soluble Antibodies
for Screening IGF Repetition**

a. Four hundred microliters of 2xYT-AG media were added to
each cluster tube (in a rack of 96 tubes in a microtiter format, Costar
10 #4411).

b. The media in cluster tubes were inoculated by transferring the
individual well-isolated colonies from the 2xYT-AGN plates using sterile
toothpicks; the cluster tubes were then incubated overnight at 30°C with
shaking (250 rpm). The array of bacterial cultures in cluster tubes
15 constitutes the Master Plate.

c. The next day, the Master Plate was duplicated by transferring
40 µl of the saturated culture from each tube of the Master Plate to 400 µl of
2xYT-AG medium in a new set of cluster tubes. The new array of
duplicated cultures in the microtiter plate format was labeled S1.

d. Plate S1 was incubated for 2 h at 30°C with shaking (250 rpm),
20 and then centrifuged at 1,000 X g for 20 min at RT in a centrifuge equipped
with microtiter plate adapters.

e. The supernatant was carefully removed from each cluster tube
and discarded to an appropriate waste container. Four hundred microliters
25 of the 2xYT-AI medium (no glucose added) was added to each tube in plate
S1, and the plate was incubated overnight at 30°C with shaking (250 rpm).

f. Plate S1 was centrifuged as described above, and 320 µl of
each supernatant (containing the soluble recombinant antibodies) was
carefully transferred to a corresponding tube in a new set of 96 cluster
30 tubes. The new plate was labeled S2.

by phage preparation according to the Protocol Preparation of Phage described below.

Protocol: Preparation of Phage

The general protocol for phage preparations used to prepare phage displayed rVabs is described below.

1. Phagemid-containing TG1 cells were grown to $OD_{600} = 0.5$ in 2xYT-AG media at 37°C with shaking (250 rpm).
2. M13K07 helper phage were then added (at $MOI = 20$), and the cells were incubated for 1 h at 37°C with gentle shaking (150 rpm).
- 10 3. Following infection, cells were pelleted by centrifugation at 1,000 g for 20 min and the supernatant containing the helper phage were discarded.
4. The cell pellet was resuspended in the initial culture volume in 2xYT-AK and grown overnight at 30°C with shaking (250 rpm).
- 15 5. The cells from the overnight culture were pelleted at 3,000 g for 30 min at 4°C and the supernatant containing the phage was recovered.
6. The supernatant was adjusted to contain 4% PEG, 500 mM NaCl and chilled on ice for 1 h. The precipitated phage was pelleted by centrifugation at 10,000 x g for 30 min. The pellet was resuspended in
20 MPBS.

Example 17: Expression and Characterization of IR Binding rVab Clones

A. Infection of *E. coli* HB2151 Cells

- a. To prepare the log-phase cells, 2xYT media was inoculated
25 with *E. coli* strain HB2151 cells (genotype) from a fresh minimal medium plate, and the cells were grown to $OD_{600} = 0.5$ at 37°C with shaking (250 rpm).

values indicate readings taken at OD₄₀₅ which were normalized as percent control. The control fusion protein MBP-447 contains a peptide that binds the growth hormone receptor. Peptides in bold type contain cysteine residues. Underlined and in bold are values which are $\leq 54\%$ of control values.

Example 16: Biopanning the rVab Library

The same rVab library described in Example 5 and panned for members that bound IGF-1R was also panned for members that bind IR. Human insulin receptor was diluted to 1 mg/ml in 50 mM sodium carbonate buffer, pH 9.5. One hundred microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. The wells were then blocked by adding 100 μ l of MPBS to each well and incubating at RT for 1 h.

The phage were incubated with MPBS for 30 min at RT, then 100 μ l of the phage solution were added to each well and incubated for 2 h at RT. In the first round, the input phage titer was about 10^{13} cfu/ml. The input phage titer was about 10^{11} cfu/ml in subsequent rounds.

The wells were washed 13 times with 200 μ l/well of MPBS, then washed once with PBS (200 μ l/well). The bound phage were eluted by adding to each well 100 μ l of 20 mM glycine-HCl, pH 2.2. After 30 s, the phage was transferred to an Eppendorf tube and the solution was neutralized by adding 50 μ l of 1 M Tris-HCl, pH 8.0, per volume from each well.

TG1 cells were grown to the mid-log phase (OD₆₀₀ = 0.5). Equal volumes of the TG1 cell culture and the neutralized phage solution were mixed together, incubated for 1 h at 37°C without shaking, and then plated onto two 24 cm x 24 cm 2xYT-AG agar plates. The next morning, cells were removed by scraping the surface of the agar plates, and were then suspended in 24 ml 2xYT and stored in 10% glycerol at -80°C.

The input phage for the subsequent rounds of biopanning was prepared by growing 100 μ l of the cells from these frozen stocks, followed

were not able to compete to a significant extent any of the phage-displayed peptides from any of the groups. It should be noted that the corresponding identical phage from Group 1 was not tested. The data support the conclusion that the cysteine-containing peptides bind to a contact site (Site 2) which is different than the contact site (Site 1) required for the consensus containing peptides (Group 1, (Formula 1 motif)) to bind the insulin receptor.

TABLE 11

Phage Displayed		Group 1		Group 2		Group 4	Group 7	Control
Peptides		MBP-E7 1.6 μ M	MBP-H8 1.6 μ M	MBP-A7 (20A4) 5 μ M	MBP-D8 2 μ M	MBP-D10 4 μ M	MBP-F8 2.8 μ M	MBP-447 14 μ M
Group 1	B8	265	264	329	267	274	240	299
	20 D3	196	196	250	170	218	208	186
Group 2	D8	138	135	<u>53</u>	<u>54</u>	129	111	160
	A7 (20A4)	133	103	<u>28</u>	<u>54</u>	125	<u>21</u>	116
Group 3	20 E2	80	106	100	69	84	161	100
Group 4	A2	92	92	88	74	105	98	79
	D10	92	60	<u>20</u>	<u>20</u>	<u>36</u>	<u>20</u>	117
Group 6	F2	91	97	88	83	92	83	101
	E8	86	75	164	99	94	86	110
Group 7	F8	99	93	<u>44</u>	63	82	<u>43</u>	138
Group 8	40 A2	80	74	118	84	95	80	90

Data reported in the table above was obtained as follows: IR was coated on a 96-well plate with 50 μ l of 2 ng/ μ l IR and incubated overnight at 4°C. The wells were then blocked with MPBS for 1 h. The fusion proteins (mixed #1:5 with MPBS) were added to the wells and incubated at RT for 30 min. An equal volume of phage (displaying various peptides from each of the groups) was then added and incubated for 1.5 h. The control well contained only phage and an equal volume of buffer. The plate was washed 3 times in PBST and then incubated with HRP/anti-M13 conjugate for 45 min. The plate was washed again and then the ABTS substrate added. The

Exempl 15: Insulin Receptor Competition ELISA Using Phage Displayed Peptides and MBP-Peptide Fusion Proteins

To determine whether the binding sites (contact sites) on the insulin receptor for the various peptides are similar, the purified fusion proteins
5 were used in ELISA competition experiments with phage displayed peptides from various groups. Phage-displayed peptides, which were able to bind to IR, were classified into various groups according to consensus sequences identified (see Figures 47 and 48). Peptide sequences of interest were fused to the C-terminus of MBP as previously described. The protein fusion
10 constructs were expressed as soluble proteins, purified, and the protein concentrations were determined. The purified fusion proteins were used in ELISA competition experiments with phage displayed peptides from the various groups as shown in Table 11.

As expected, the fusion proteins containing A7 (20A4), D8, D10, and
15 F8 peptides were able to compete the corresponding identical peptide sequence displayed on phage in the range of 28-54% of the control value. The fusion protein, MBP-A7, was able to significantly compete (<54%) phage-displayed peptides D8, D10, and F8. The other fusion protein from Group 2 (Formula 6 motif), MBP-D8, was able to compete A7 and D10
20 peptides displayed on phage. Furthermore, the Group 7 (Formula 4 motif) fusion protein MBP-F8 competed A7 and D10 phage displayed peptides. Figures 56A and 56B show the plotted data from Table 11. In Figure 56A, a clear pattern is seen where significant ($\leq 54\%$) competition reactions occur between fusion proteins and phage-displayed peptides which have in
25 common the presence of at least two cysteine residues (see Figures 47 and 48 for peptide sequences).

Also striking is the observation that the cysteine containing fusion proteins were not able to compete phage displayed peptides from Group 1 (Formula 1 motif), which contain the consensus (IGF A6-like) sequences
30 and are without cysteine residues (Figure 56A). In Figure 56B, the fusion proteins containing the Group 1 (Formula 1 motif) consensus sequences

Flowcell-1 was used for background binding to correct for any non-specific binding. Insulin receptor (450 nM) was injected into the flow cell and the binding of IR to insulin was measured in resonance units (RUs). Receptor bound to insulin gave a reading of 220 RU. The surface was regenerated with 25 mM NaOH. Pre-incubation of receptor with insulin in a tube at RT completely abrogated the response units to 16 RU. Thus, the system was validated for competition studies. Several maltose-binding fusion proteins, peptides and rVabs were pre-incubated with insulin receptor before injecting over the insulin chip for competition studies. The decrease in binding/resonance units indicates that several MBP-fusion proteins can block the insulin binding site. The results are shown in Tables 9 and 10. The amino acid sequences referred to in the tables are identified in Figures 47 and 48, except the 447 and 2A9 sequences, which are shown below.

TABLE 9: BIAcore Results—Fusion Proteins Compete for Binding to IR

	Incubation Mixtures	Result (RUs)	Sequence Type
Controls	Insulin Receptor (IR) 450 nM	220	Positive Control
	Insulin (8.7 μ M)	16	Negative Control
MBP Fus. Prots.	A7 (20A4)-MBP (4.1 μ M) + IR	43	Formula 6 Motif
	D8-MBP (1.6 μ M) + IR	56	Formula 6 Motif
	D10-MBP (3.4 μ M) + IR	81	Formula 11 Motif
	447-MBP (11.5 μ M) + IR	195	hGH Pept. Fus.
	MBP (13 μ M) + IR	209	Negative Control

TABLE 10: BIAcore Results—Synthetic peptides compete for binding to IR

Incubation Mix	% Binding	Result (RUs)	Sequence Type
IR	100	128	Positive control
IR + 20D1	41	51.8	Formula 1 Motif
IR + D8	33	41.6	Formula 6 Motif
IR + 20C11	38	49	Formula 2 Motif (bkg high)
IR + H2	27	34.6	IGF (phosphorylated band)
IR + 2A9	100	128	IGF (bkg high)
IR + 20A4	33	41.8	Formula 6 Motif
IR + p53wt	97	124.5	P53 wild type

The concentration of each peptide was about 40 μ M and the concentration of IR was 450 nM. The 447 peptide sequence is: LCQRLGVGWPGWLSGWCA. The 2A9 peptide sequence is: LCQSWGVRIGWLTGLCP.

	Glucose	3.00
	(NH ₄) ₂ SO ₄	5.00
	MgSO ₄ • 7H ₂ O	0.25
	KH ₂ PO ₄	3.00
5	Citric Acid	3.00
	Peptone	10.00
	Yeast extract	5.00
	pH 6.8	

- 10 The culture was grown at 700 rpm, 37°C until the glucose from the medium was consumed (OD₆₀₀ = ~6.0 – 7.0). Expression of the fusion protein was induced by the addition of 0.3 mM IPTG and the culture was grown for 2 h in fed-batch mode fermentation with feeding by 50 % glucose at a constant rate of 2 g/l/h. The cells were removed from the medium by
- 15 centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

- 20 The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent Triton X-100. After removal of cell debris by centrifugation, the soluble proteins were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The MBP fusions were initially purified either by amylose affinity chromatography or by anion exchange chromatography. Final
- 25 purification was performed using anti-E-Tag antibody affinity columns (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with Elution buffer (100 mM glycine, pH 3.0). The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard
- 30 protocols.

For BIAcore analysis of fusion protein and synthetic peptide binding to insulin receptor, insulin (50 µg/ml in 10 mM sodium acetate buffer pH 5) was immobilized on the CM5 sensor chip (Flowcell-2) by amine coupling.

spin columns (QIAGEN) and electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 μ l of *E. coli* strain ER2508 (RR1 *lon::miniTn10(Tet^r) (malB) (argF-lac)U169 Pro⁺ zjc::Tn5(Kan^r) fhuA2*) electrocompetent cells (New England Biolabs). Immediately after the pulse, 1 ml of pre-warmed (40°C) 2xYT medium containing 2 % glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were plated onto 2xYT-AG plates and grown overnight at 37°C. Sequencing confirmed the clones contained the correct constructs.

10 **B. Small-Scale Expression of Soluble MBP-Peptide Fusion Proteins**

E. coli ER2508 (New England Biolabs) carrying the plasmids encoding MBP-peptide fusion proteins were grown in 2xYT-AG at 37°C overnight (250 rpm). The following day the cultures were used to inoculate media (2x YT containing-G) to achieve an OD₆₀₀ of 0.1. When the cultures reached an OD₆₀₀ of 0.6, expression was induced by the addition of IPTG to a final concentration of 0.3 mM and then cells were grown for 3 h. The cells were pelleted by centrifugation and samples from total cells were analyzed by SDS-PAGE electrophoresis. The production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia).

C. Large-Scale Expression of Soluble MBP-Peptide Fusion Proteins

25 *E. coli* ER2508 carrying plasmids encoding the MBP-peptide fusion proteins were grown in 2xYT-AG media for 8 h (250 rpm, 37°C). The cultures were subcultured in 2xYT-AG to achieve an OD₆₀₀ of 0.1 and grown at 30°C overnight. This culture was used to inoculate a fermentor with medium of following composition (g/l):

Example 14: Peptide Fusions to the Maltose Binding Protein - Construction, Purification and Characterization of the Binding to the Insulin Receptor

A. Cloning

5 The transfer of interesting peptide sequences from phage display to display as maltose binding protein (MBP) -fusions is desirable for several reasons. First, to obtain a more sensitive affinity estimate, the polyvalency of phage display peptides should be converted to a monovalent system. For this purpose, the peptide sequences are fused to MBP that generally exists
10 as a monomer with no cysteine residues. Second, competition experiments can be carried out with the same or different peptides, one phage displayed and the other fused to MBP. Lastly, purified peptides can be obtained by cleavage of the fusion protein at a site engineered in the DNA sequence.

Figure 55 shows a schematic drawing of the MBP-peptide construct.
15 In the construct, the N-terminus of the peptide sequence is fused to the C-terminus of the MBP. Two peptide-flanking epitope tags are included, a shortened-FLAG at the N-terminus and E-Tag at the C-terminus. The corresponding gene fusion was generated by ligating a vector fragment encoding the MBP in frame with a PCR product encoding the peptide of
20 interest. The vector fragment was obtained by digesting the plasmid pMAL-c2 (New England Biolabs) with *EcoRI* and *HindIII* and then treating the fragment with shrimp alkaline phosphatase (SAP; Amersham). The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN). The 20-amino acid peptide sequences of
25 interest were initially encoded in the phage display vector pCANTAB5E (Pharmacia). To obtain these sequences, primers were synthesized which anneal to sequences encoding the shortened FLAG or E-Tag epitopes and also contain the required restriction enzyme sites *EcoRI* and *HindIII*. PCR products were obtained from individual phage clones and digested with
30 restriction enzymes to yield the insert fragment. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick

B. Results

At high insulin dosage, all clones, except F4, G7, and H4 # (not shown), were inhibited; B8 showed the best inhibition, >50%. The apparent lack of binding of F4 (group 1) might be due to the insufficient level of phage present. G7 is a Fc binding phage is should not by inhibited by insulin. H4 is suspected to be a plastic-binding phage. The results are presented in Figure 54.

C. Conclusions

Insulin competition with a representative member from each group indicated that almost all of the groups competed with insulin; only the "plastic binders" and Fc binding phage did not compete. Different degrees of inhibition by these peptides (phage) imply that the peptides recognize different epitopes on or in the close proximity of the receptor active site.

Example 13: Synthetic Peptide 20A4 Competition Results

This experiment was performed as in Example 12. The 20A4 peptide (D122) starting concentration was 58 μ M.

Results. The results are included in Table 7. The peptide 20A4 (D122) (A7) competes with Group 2 members (Formula 6 motif), Group 4 member (miscellaneous) D10, and Group 7 member (Formula 4 motif) F8 (D124). There is a partial inhibition of Group 6 member F2. The data is consistent with the conclusion that the site for 20A4 binding is different from the site for Group 1, Group 3, and Group 6.

between the two receptors occurs despite negative results of many of these same phage-displayed peptides.

- 5 b. Although the C1 peptide is the most potent inhibitor of phage binding, C1 peptide loses much of its potency advantage over the other peptides binding IR instead of IGF-1R. In addition, A6 gains potency when binding to IR relative to the other peptides. Combined, this suggests that the adjacent surfaces to this active site of the receptors are sufficiently different that peptides and small organic molecules specific for either receptor can be found.
- 10 c. The Hill Coefficient of the peptides binding to IGF-1R is always 1.5 to 2-fold higher than the same phage and peptide binding to IR.

Example 12: Competition of Phage Binding with Insulin

Many different peptides isolated from the random peptide libraries were tested for the ability to compete the natural ligand insulin. Clones
15 tested were B8 (D103) (Formula motif 1), F4 (Formula motif 1), A7 (D122) (20A4) (Formula motif 6), D8 (D123; data not shown) (Formula motif 6), C6 (Formula motif 2), E8 (Formula motif 10), H4 (group 5; data not shown), A4 (group 6), G8 (group 7), G7 (Fc binder). H4 most likely binds non-specifically to the material from which the microtiter plate is made.

20 A. Insulin Competition Procedure

Receptors were coated at 100 µg/ml, 50 µl/well. After blocking with MPBS and washing 3x with PBST, insulin was added in the presence of 0.1% Tween-20 at 2 µM, 100 nM, and 5 nM for 15 min before the addition of IR binding phage. The final concentration of insulin was 1 µM, 50 nM and
25 2.5 nM. Reaction was incubated at RT for 1 h and wells were washed 3x with PBST (PBS with 0.05% Tween-20). Anti-M13 HRP was added and incubated for 1 h at RT. Wells were washed 3x with PBST before the addition of ABTS. Plates were read at 405 nm.

the serial dilution until Column 2. Fifty microliters were discarded from Column 2 to maintain 100 μ l per well. Column 1 was reserved as no peptide wells. The starting working concentrations for each peptide was: H2 – 50 μ M; H2C – 100 μ M; C1C – 100 μ M; D2C – 100 μ M; E4 – 33.3 μ M; C1 – 50 μ M; A6 – 100 μ M; and p53 – 100 μ M.

B. IGF-1R Peptide Competition

An experiment was designed to ascertain whether IGF-1R peptides have the ability to compete phage that bind to IR. Competition will occur in either IR- or IGF-1R-coated wells. The IGF-1R peptides H2, H2C, C1C, D2C, E4, C1, and A6 were tested for competition with two separate phage. The first, 20D3, (Figures 51A, 51C) is a phage discovered during panning of IR, but is also positive for binding to IGF-1R. The second, H2, (Figures 51B, 51D) is a phage found during panning of the IGF-1R, but is also positive for binding to IR. A p53-like peptide that binds to MDM2 was used as a negative control.

The Hill Plot data are provided in Table 8 below, and presented graphically in Figures 52A-52D.

TABLE 8: Hill Plot Data

Pept.	IGF Receptor						Insulin Receptor					
	20D3 Phage			H2 Phage			20D3 Phage			H2 Phage		
	n	K _d	r ²	n	K _d	r ²	n	K _d	r ²	n	K _d	r ²
H2	1.29	4958	0.991	1.21	9812	0.979	1.07	1133	0.978	0.71	762	0.981
H2C	0.81	5055	0.975	1.02	3720	0.987	1.03	564	0.976	0.62	480	0.926
C1	1.37	19	0.988	0.96	40	0.976	0.83	324	0.999	0.46	132	0.922
C1C	1.32	13475	0.990	1.00	34198	0.945	0.70	1190	0.988	0.53	532	0.956
D2C	1.50	12454	0.995	1.34	33124	0.999	0.81	2491	0.995	0.96	2964	0.983
E4	1.53	6522	0.995	1.11	5868	0.961	0.79	1435	0.979	0.71	387	0.994

C. Observations and Conclusions

a. These peptides can bind to IR and inhibit binding of phage found by either panning IR (20D3) or IGF-1R (H2). This crossover event

Exempl 11: Cross-Reactivity Studies

Phage ELISA experiments show that the IGF-1R peptides H2 and E4 have detectable binding to IR while expressed as a phage fusion. Other IGF-1R-specific peptides such A6, C1, B6, and JBA5 do not have detectable
5 binding to IR when expressed as phage.

A. Experimental Procedures

Phage Production. Each phage culture was started by the addition of 40 µl of the MASTER stock to 20 ml 2xYT-AG in 50 ml centrifugation tubes. Cultures were incubated at 37°C until OD₆₀₀ ~0.6-1.0. M13K07 helper
10 phage were added to a concentration of ~5 x 10¹⁰ cfu/ml and incubated at RT for 30 min. The cultures were centrifuged at ~2500g and 4°C for 20 min. The bacterial pellet was resuspended in 20 ml 2xYT-AK and incubated O/N at 37°C. The culture was centrifuged at ~2500 x g and 4°C for 20 min. The supernatant was transferred to new 50 ml centrifuge tubes

15 *Phage ELISA.* Each well of the Nunc-Immuno™ plates with the MaxiSorp™ surface were coated with 50 µl of 2 ng/µl either IR or IGF-1R in PBS O/N at 4°C. The wells were blocked with 200 µl of 2% (w/v) Carnation non-fat dry milk in PBS for 1.5 h at RT. Phage were added at 100 µl per well. Peptides were added as noted below and allowed to incubate at RT
20 for 3 h. The plates were washed 3X with PBST. A solution of 1:3000 diluted HRP:Anti-M13 Conjugate at 100 µl per well of was added for 1 h. Following a repeat of the washing, 100 µl of ABTS was added for 15-30 min. The OD₄₀₅ was measured using a SpectraMax 340 Microplate Spectrophotometer.

25 *Peptide Competition.* Peptide Competition Curves were produced during the phage ELISA across rows in triplicate. The stock synthetic peptide solution was added to Column 12 so that the total volume totaled 150 µl (additional phage solution was added when necessary). A serial
30 dilution was made by transferring 50 µl from Column 12 into 100 µl in Column 11, 50 µl from Column 11 into 100 µl in Column 10, and continuing

Observations and Conclusions

1. The C1 and B6 peptides bind to IR. The C1 and B6 peptides expressed as phage-displayed peptides are negatively charged.
2. Groups 1, 3, and 6 (Formulas 1, 2 and 10, respectively), appear to be inhibited by both the C1 and B6 peptides when binding to IR and IGF-1R. All three groups behave with similar characteristics and similar affinities. They all bind to a common site, (Site 1) as shown by competition data.
3. Group 2 (Formula 6 motif) phage clones have different properties despite their sequence similarity. The phage 20A4 is an IR-specific clone. Its binding to IR is not inhibited by C1 or B6 peptides and therefore binds to Site 2. The phage D8 binds to both IR and IGF-1R. Inhibition by C1 peptide and B6 peptide occurs only when binding to IGF-1R. D8 is more sensitive to C1 and B6 peptide inhibition than Group 1, 3, and 6, suggesting an allosteric competition.
4. Some phage appear to have a plastic-binding component (binding to the wells of microtiter plates) in their sequences when high amounts of phage are used. The phage A2, D9-2, H4, 40F10, 40A2, and 40H4 have a significant background to their signals. With the exception of 40H4, all signals increase over this background signal in the presence of IR. The signals for phage A2 and D9-2 also increase over background for IGF-1R. It should be noted the phage for the IGF-1R binder B6 shows this similar characteristic.
5. The Group 2 phage 20A4 and Group 4 phage D10 are specific for IR – there is no detectable binding to IGF-1R. D10 may be inhibited by C1 peptide to a small extent.
6. The phage for Group 7, F8 (Formula 4 motif) has characteristics similar to Group 2, D8 (Formula 6 motif). This clone binds to both IR and IGF-1R, but the C1 and B6 peptides only affect D8 binding when bound to IGF-1R. F8 is more sensitive to C1 and B6 peptide inhibition than Group 1, 3, and 6, (Formula 1, 2 and 10 motifs, respectively).

TABLE 7: Phage Characterization Summary

	Absorbance Values			IR Competitions		sIGF-1R Competitions	
	NFM	sIGF-1R	IR	C1	B6	C1	B6
Group 1							
20D3	0.09	2.26	1.29	Y	Y	-	
B8	0.10	2.55	1.30	Y	Y	-	
Group 2							
20A4	0.15	0.21	1.61	N	N	-	-
D8	0.09	2.19	1.42	N	N	Y	Y
Group 3							
20E2	0.11	2.15	1.01	Y	Y	-	
Group 4							
D10	0.12	0.14	0.73	N*	N	-	-
A2	1.35	2.00	1.79	N	N	N	
Group 5							
D9-2	1.02	2.53	1.64	N	N	-	-
H4	1.16	1.14	1.41	N*	N	-	-
Group 6							
E8	0.10	2.00	1.34	Y	Y	-	
F2	0.09	2.08	1.43	Y	Y	-	
Group 7							
F8	0.14	2.06	1.49	N	N	Y	Y
Group 8							
40A2	0.56	0.55	1.90	Y*	Y	-	-
40H4	0.75	0.83	0.84	-	-	-	-

NFM = Non-fat milk

- 5 C1 peptide (D112) has the FYX₃WF Formula 1 motif and an amino acid sequence of DYKDCWARPCGDAANFYDWFVQQASKK

B6 peptide has the FYX₈X₉LX₁₁X₁₂L Formula 2 motif and an amino acid sequence of WNTVDPFYHKLSELLREKK

concentration of the B6 peptide was 68 μ M for both receptors. For IR, the starting concentration for the C1 peptide was 48.5 μ M. Only 2 μ l of the C1 peptide were added to Row A of wells containing IGF-1R. Therefore, the starting concentration was 4.9 μ M. The volume was maintained by the addition of 18 μ l of the phage solution to Row A.

Natural Ligand Competition. The "Phage First" experiments were performed by adding 10 μ l of 5.5 μ M, 550 nM, or 55 nM insulin or IGF-1 in PBS to phage-containing wells in the phage ELISA. The working concentrations were 500 nM, 50 nM, and 5 nM. The volume of no ligand wells was maintained by the addition of 10 μ l PBS.

The "Ligand First" experiments were performed by added 50 μ l of 2 μ M, 200 nM, or 20 nM insulin or IGF-1 in PBS containing 0.5% Tween-20 to non-phage containing wells and allowed to incubate 15 min. Fifty microliters of the phage solution was then added to the wells and mixed well. The mixture was allowed to incubate for 2 h at RT and continue with the phage ELISA.

The data are provided in Table 7 and Figures 50A-50D. Sequences were confirmed on all clones by DNA sequencing.

Example 10: ELISA Analyses of Phag

This series of experiments was designed to help characterize the different groups of consensus sequences found during the biopanning of IR. Phage were prepared from each group (two unique sequences each were attempted). Each phage was bound to insulin receptor and competition experiments were performed.

Phage Production. Each phage culture was started by the addition of 30 μ l of the master stock to 20 ml 2xYT-AG in 50 ml centrifugation tubes. Cultures were incubated at 37°C until OD₆₀₀ ~0.6-1.0. M13K07 helper phage were added to a concentration of $\sim 5 \times 10^{10}$ cfu ml⁻¹ and incubated at RT for 30 min. The cultures were centrifuged at $\sim 2500g$ and 4°C for 20 min. The bacterial pellet was resuspended in 30 ml 2xYT-AK. The culture was transferred into 250 ml bottles and incubated O/N at 37°C. The culture was centrifuged at $\sim 2500g$ and 4°C for 20 min (in 50 ml centrifuge tubes). The supernatant was transferred to new 50 ml centrifuge tubes.

Phage ELISA. Each well of the Nunc-ImmunoTM plates with the MaxiSorpTM surface were coated with either 50 μ l of 2 ng/ μ l either IR or sIGF-1R in PBS overnight at 4°C. The wells were blocked with 200 μ l of MPBS for 1.5 h at RT. Phage were added at 100 μ l per well. Peptides were added as noted below and allowed to incubate at RT for 3 h. The plates were washed 3 times with PBST. A solution of 1:3000 diluted HRP:Anti-M13 conjugate at 100 μ l per well of was added for 1 h. Following a repeat of the washing, 100 μ l of ABTS was added for 15-30 min. The OD was measured using a SpectraMax 340 Microplate Spectrophotometer (Molecular Devices) at 405 nm.

Peptide Competition. Competition of phage displayed peptides by the addition of soluble peptides was carried out using the phage ELISA as described above. Twenty microliters of the stock synthetic-peptide solution was added to Row A. A series of 20 μ l into 100 μ l dilutions were performed until Row G. Twenty microliters were discarded from Row G to maintain 100 μ l per well. Row H was reserved as no peptide wells. The starting

formation and glycosylation patterns should mimic the wild-type receptor. The details of this recombinant protein construct are described in Bass *et al.* (1996).

In panning with the peptide library, the IR was immobilized directly
5 onto a protein-binding plastic surface, and four rounds of panning and enrichment were carried out. Analysis of phage clones from rounds three and four showed that 114 of the 216 clones from the 20mer random peptide library and 17 of the 120 from the 40mer random peptide library bound to IR (Figures 1A, 1B, 2A, 2C, 4A, 6A, 10A and 10B). Of those clones tested
10 competitively against insulin for receptor binding, all were blocked by the presence of natural ligand. This result indicated that these phage clones and insulin bind to the same site (or at least overlapping sites) on IR.

Sequence analysis of several clones shows that there are several distinct populations, designated as Groups 1 through 8 (Figures 1-8)
15 (Figures 47 and 48). Several of the peptides based on the sequences for these groups have been chemically synthesized for subsequent testing. Group 1 (Formula 1 motif) peptides contain the consensus sequence FYxWF, and are believed to be agonistic in cell-based assays. Group 2 (Formula 6 motif) is composed of two peptides having a consensus
20 sequence VYGR and two cysteine residues each. Thus, Group 2 peptides are capable of forming a cyclic peptide bridged with a disulfide bond. Group 3 (Formula 2 motif) peptides comprise the preferred consensus sequence F-Y-x-A/G-L/I-x-x-L (A/G denotes the alanine or glycine residue, and L/I denotes the leucine or isoleucine residue). Certain Group 3 peptides exhibit
25 agonistic activity in cell-based assays (Figure 49). Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature insulin.

Group 7 (Formula 4 motif) is composed of two exemplary peptides which do not have any significant sequence homology, but have two
30 cysteine residues 13-14 residues apart, thus being capable of forming a cyclic peptide with a long loop anchored by a disulfide bridge.

input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2-3 h at RT. The wells were then quickly washed 13 times with 300 μ l/well of PBS containing 0.5% Tween-20 (PBST). Bound phage were eluted by incubation with 150 μ l/well of 50 mM glycine-HCl, pH 2.0 for 15 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0. An equal volume of log-phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A total of 216 clones from the 20mer library and 120 clones from the 40mer library were picked at random from the third and fourth rounds of panning and screened for IR binding activity. DNA sequencing of the clones revealed the abundance of sequences as summarized in Figures 1A, 1B, 2A, 2C, 10A and 10B.

B. One-Day Panning Procedure

Log phase TG1 cells were infected with the eluted phage, amplified in the 2xYT medium for 1 h at 37°C prior to the addition of helper phage, ampicillin and glucose (2% final concentration). After incubation for 1 h at 37°C, the cells were spun down and resuspended in the 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. The overnight phage was then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity.

To isolate specific binders, each library was panned against a soluble form of the human IR. This IR is composed of the extracellular domains of both the α and β chains of the natural receptor, as well as the constant domain from immunoglobulin Fc, retaining the β - α - α - β structure described above. Because the IR is expressed in a eukaryotic system, disulfide bond

TABLE 6[illegible]

25 Example 9: Panning the Insulin Receptor

A standard method was used to coat and block all microtiter plates. IR (prepared according to Bass *et al.*, 1996) was diluted to 2 µg/ml in PBS. Fifty microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with a solution of 2% non-fat milk in PBS (MPBS) at RT for at least 1 h.

A. Two-Day Panning Procedure

Eight wells coated with IR were used for each round of panning. One hundred microliters of phage were added to each well. For the first panning round, the input phage titer was 4×10^{13} cfu/ml. For subsequent rounds, the

Example 8:

A composite of amino acid residues observed in sequences of random 20mer, 40mer and A6 (Formula 1) clones is illustrated below:

	A6 CORE	N	F	Y	D	W	F
5		D6				A	
		E				E9	
		G6				G2	
		H3				Q4	
		K				R	
10		P				S	
		Q8					
		S					
		T					
		V					
15							

A summary of preferences for A6 residues is shown in Table 5 below. An illustration of residues which are characteristic of IGF-1R binding sequences (above parental sequence) and those which are not typically associated with binding sequences (below parental sequence). Table 6.

20

TABLE 5

	X1	X2	X3	X4	X5
1(N)	2(F)	3(Y)	4(D)	5(W)	6(F)
no aromatics; no large aliphatics; no C; no P			no hydrophobics, except tiny; no C; no P		

TABLE 3

				*	*		*		*			
	B6 CORE	D37	P34	F54	Y54	H12	K15	L46	S16	E27	L53	L30
5		E	A			A4	A10	I7	A5	A6	M	A6
		G3	D4			D7	G7	M	D3	D3		I
		K4	E			E9	I		E4	G2		K
		R2	G10			G	L8		F5	K		S2
		S5	L			K3	M3		G4	L		T
10		T	Q			L2	N		H	Q3		V13
		V	S			M	Q		L6	R6		
			T			N	R5		M	S4		
						Q7	T		N	V		
						R4	V		Q2			
15						S	W		R2			
						T			T2			
						V			Y2			

Based on the substitutions observed above, the following preferences
 20 shown in Table 4 are preferred for substitutions in the amino acid sequence
 of Formula 2 for binding to IGF-1R.

TABLE 4

25X98	X99	X6	X7	X8	X9	X10	X11	X12	X13	X100
1(D)	2(P)	3(F)	4(Y)	5(H)	6(K)	7(L)	8(S)	9(E)	10(L)	11(L)
no aromatics; no large aliphatics; no c	no aromatics; no c; no + charged			no aromatics; no C; no P; no I	no aromatics, except W; no - charged; no C; no P		no C; no P	no aromatics; no C; no P		no aromatics; no aliphatics; no C; no P

random 20mer library were analyzed in a phage ELISA to identify binders. The DNA of binders was then determined. The results from both libraries show that positions other than positions 3, 4, 7 and 10 as described above can vary relatively at ease (see tables below), while variability at positions 3, 4, 7 and 10 is much more restricted. The results from the B6-2 library show that the restricted core residues were maintained in all binders except one, which happened only in one instance, L (position 7) can be substituted by another hydrophobic residue, M, at that position. The result from the random 20 library panning revealed that another aliphatic amino acid residue, I can substitute for L at position 7. In addition, the restricted residue at position 10 (L) can also be substituted with amino acid residue M. Thus, 2 of the previously identified restricted residues (L at positions 7 and 10) are not absolute, even though L is preferred at these positions. It should be noted that the failure to observe a substitution at a particular residue position does not necessarily indicate that substitutions cannot be made without losing activity, rather such an absence of substitution is indicative of a preference or an aversion for substitution. The findings are summarized below:

B. Results

Combined results from binding clones isolated from B6-2 (doped core) and random 20 libraries of the Formula 2 motif are shown below in Table 3. Sequences from 25 clones from B6-2 and 29 clones from the random 20mer library were analyzed. Numbers adjacent the amino acid residues represent the frequency with which a specific amino acid was observed at the corresponding position.

receptor pairs to be used in a site directed competition binding assay wherein IGF-1R can be used as one member of the pair, with the peptide or a rVab as the other member. Labeling of each member, and detection of pair formation, using either member in radioactive or nonradioactive labeled forms, is possible by a variety of methods known to those skilled in the art of building competition binding assays. This assay provides a high throughput screening assay to identify small organic molecules which bind to the active site of IGF-1R.

Example 7: Phage Library B6-2

10 This library was designed based on the "core" sequence of the Class I binders Site 1(B6) which posses antagonistic activities in a cell proliferation assay. The core sequence was determined as DPFYHKLSEL, where the residues F (position 3, X₆ of Formula 2), Y (position 4, corresponding to X₇ of Formula 2), L (position 7, corresponding to X₁₀ of Formula 2) and L (position 15 10, corresponding to X₁₃ of Formula 2) were the only residues observed at those positions. The purpose of this library was to test the possibility that some binders will show deviations from the core sequence, especially at the positions where substitutions had not previously been observed. The library was therefore made from doped oligonucleotides so that, on average, half of 20 the amino acid residues were altered per peptide. The library was made as described in the original B6 library, i.e., synthetic oligonucleotides were first amplified in a PCR reaction. The resultant products were cloned into pACANTAB5E (Pharmacia) via *Sfi*I and *Not*I restriction sites as previously described. Over 10¹⁰ different clones were obtained in the final library.

25 **A. Random 20mer Library**

1. Panning with the B6-2 and Random 20mer Libraries

The libraries were affinity selected against IGF-1R. 96 clones from round 3 of panning from B6-2 library and 96 clones from round 4 from the

Exempl 6: Agonistic and Antagonistic Activity of IGF-1R-Binding Peptides

Agonistic and antagonistic activities of the IGF-1-specific peptides were tested in FDCP2 cells (NIH) which express IGF-1R. The cell line
5 requires either IL-3 or IGF-1 for growth, and the cells were maintained in RPMI 1640 medium containing 15% FCS (fetal calf serum). Agonism activity assays were performed in a total volume of 100 μ l in 96 well plates (flat bottom). Cells were seeded at 30,000 cells/well in 50 μ l RPMI 1640 (without IL-3) medium containing 15% FCS in triplicate wells. To each well,
10 50 μ l of a solution containing either IGF-1, rVabs or peptides at different concentrations was added, followed by incubation for 42 h in a CO₂ incubator at 37°C.

Assays to measure the antagonistic activity were performed in a total volume of 100 μ l in 96 well plates. An IGF-1-specific peptide, rVab or an
15 appropriate control was added to wells containing 0.003 μ M of human IGF-1 and incubated at 37°C for 18 h in CO₂ incubator. Proliferation assays were performed using WST-1 reagent. The WST-1 tetrazolium salt (slightly red) is cleaved to formazan (dark red) by the succinate-tetrazolium reductase system, which is active only in viable cells. An increase in the number of
20 cells results in an increase in the overall activity of the dehydrogenase which results in a higher absorbance at 450 nm. Ten microliters of WST-1 reagent was added to each well and the plates incubated for 1-4 h at 37°C. Proliferation was measured by absorbance at 450 nm. Both 5.3 and 5.4 peptides showed an agonistic activity at the 10 μ M concentration (Figure
25 23). Peptides 5.1 and 5.2 showed a significant antagonistic activity in the 3-30 μ M concentration range (Figure 22). Control peptide showed no antagonistic activity at the concentrations tested.

The results described demonstrate the feasibility of both the chemical synthesis of and construction of a recombinant expression vector to make
30 sufficient soluble peptide (free or as fusion with some carrier protein) or rVab for testing agonist and antagonist activities. The results provide peptide-

twice background) of 0.05 fmol bIGF-1. The ability of this assay format to detect specifically bound bIGF-1 (or bPeptides) to IGF-1R coated wells was determined.

8. Elisa Analyses

5 ELISA was performed on selected rVabs. We found that the native IGF-1 ligand inhibits the binding of peptide 5.1 (the sequence of which originates from the phage clone B6) as shown in Figure 43. The detection of the peptide involved a sandwich configuration with the Eu-labeled streptavidin. It was determined that the binding of Eu-labeled rVab 43G7 to
10 IGF-1R is inhibited by IGF-1 with an IC_{50} of approximately 2 nM, as shown in Figure 44. The binding of the biotinylated peptide 5.1 is inhibited by rVab 43G7 with an IC_{50} of about 10 nM (Figure 45), indicating that both the peptide and rVab bind to the same site on the IGF-1R molecule.

Figures 46A-46D demonstrates the binding properties of the 43G7
15 antibody. The binding of the Eu-labeled 43G7 antibody is competed by peptide 5.1 (clone B6) (Figure 46A) and by the non-labeled 43G7 (Figure 46B), as well as by rVab 39F7 (Figure 46C) and rVab 1G2P (Figure 46D). The sequences of rVabs 1G2P and 39F7 are provided in Figure 35 and Figure 36, respectively.

20 C. Conclusions

The above results support the use of this assay procedure as a high throughput screen for agents, with affinities for sites on the human IGF-1R which bind IGF-1. The studies show the IGF-1-specific peptides bind in a dose-dependent, saturable manner and are blocked from binding by agents
25 known to bind to the active site of the receptor. This competition is reproducible and easily quantified. Furthermore, the TRFD assay, which is automatable, is much more sensitive than is an ELISA.

labeling efficiency were determined using a Europium standard solution (Wallac).

b. Assay Method

IGF-1R (5 $\mu\text{g/ml}$ in 50 mM NaHCO_3) was coated onto low-
5 fluorescence MaxiSorp (Nunc) plates (100 $\mu\text{l/well}$) overnight at 4°C. The
plates were blocked with PBS containing 2% non-fat milk and 0.05% BSA
for 2 h at RT, followed by three PBS washes. For competitive ELISA, serial
dilutions of unlabelled IGF-1 (0.1 nM-100 μM) were added to the plates (100
10 $\mu\text{l/well}$) and incubated at RT for 1-2 h. 100 μl [Eu^{3+}] rVab 43G7 in Wallac's
DELFA assay buffer (100 mM Tris-HCl, pH 7.8; 150 mM NaCl; 0.5% BSA,
0.05% bovine Ig; 0.05% NaN_3 ; 0.01% Tween-20) was added and incubated
for 1.5 h at RT. The plates were then washed 5 times with TTBS (TBS
buffer containing Tween-20; Wallac) and tapped dry. Subsequently, 100 μl
15 of DELFA enhancement solution (100 mM acetone-potassium hydrogen
phthalate, pH 3.2; 15 mM 2-naphthyltrifluoroacetate; 50 mM tri(n-octyl)-
phosphine oxide; 0.1% Triton X-100) was added to each well, and the plates
were shaken for 10 min at RT. Fluorescence of each sample well was
measured at 615 nm using a DELFA 1234 fluorometer (EG&G Wallac).

The dose response of TRFD of Eu was studied in microtiter wells.
20 Detection is linear over the range 0.2 to 200 fmol with a limit of detection
(twice background) of 0.05 fmol. There are 6010 fluorescent units (FU) per
fmol of Eu. Binding and detection of Eu-SA, (4.7 mol Eu/mol streptavidin) to
wells coated with biotinylated BSA (bBSA) (6 mol biotin/mol BSA) is linear
over the entire range tested. The specific fluorescent activity of streptavidin
25 Eu-SA (with 4.7 mol Eu/mol SA) is 28 kfu/fmol and the limits of detection
(i.e., twice background) are 0.030 fmol. Coating with IGF-1R was linear up
to inputs of 200 ng/well and thereafter appeared to saturate at about 660 ng
bIGF-1 (biotinylated IGF-1) per well. This is the expected amount based on
the manufacturer's information about protein saturation densities of these
30 wells (Nunc manual). These studies show a limit of detection of bIGF-1 (i.e.,

7. Time-Resolved Fluorescence Assay

We have selected the basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for IGF-1. In the present assay, occupation of the active site of IGF-1 receptor is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IGF-1 receptor (i.e., IGF-1R:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993). We demonstrate the assay using 43G7 rVab or a biotinylated peptide. Furthermore, we show that both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IGF-1R by IGF-1.

In these assays, soluble IGF-1 receptor is coated on the surface of microtiter wells, blocked by PBS containing milk and BSA, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu^{+3} which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IGF-1R:bP bound saEu is then converted into its highly fluorescent state and detected by TRFD.

a. Preparation of [Eu³⁺]-Labeled rVab 43G7

One milligram of rVab 43G7 (the sequence is provided in Figure 34) was added to 300 nmol Eu^{3+} -chelated $\text{N}^1(\text{P-isothiocyanatobenzyl})$ -diethylenetriamine- $\text{N}^1, \text{N}^2, \text{N}^3$ -tetracetic acid (Wallac). The reaction was conducted at pH 8.5. The tube was mixed gently and placed at ambient temperature. When the reaction was complete (16 h), the sample was diluted 10-fold into the Tris-buffered saline (TBS), pH 7.5, and the separation of the labeled rVab from the unlabeled rVab and free- Eu^{3+} was achieved by using the PD-10 column. The protein concentration and

10,000 g for 15 min. The aqueous phase was discarded, and the pellet resuspended and dialyzed in PBS (phosphate buffered saline, pH 7.4) at 4° C overnight. Insoluble material was removed by centrifugation at 10,000 g, and the supernatant was filtered through a 0.22 µm membrane and purified on an anti-E-Tag antibody affinity column (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with the Elution buffer (100 mM glycine, pH 3.0). The rVab was concentrated to 1 mg/ml, dialyzed against TBS and stored at 4° C. The SDS-PAGE, Western blot analysis and N-terminal sequence analysis of the affinity purified material were performed according to standard protocols.

5. Size Exclusion FPLC Chromatography

The affinity purified rVabs were fractionated by size exclusion FPLC on a Superdex 75 HR10/30 column (Pharmacia) to determine the molecular size and aggregation state of the rVabs. For calibration of the column, High and Low Molecular Weight Gel Filtration Calibration Kits (Pharmacia) were used. Fractions from several chromatographic separations corresponding to a molecular weight of 30 kDa were pooled and concentrated to 0.7-1.0 mg/ml using Amicon XM10 membranes. Protein concentrations were determined using the BCA protein assay kit (Pierce Chemical Co., Rockford, IL).

6. BIAcore Analyses

IGF-1R was immobilized onto one flow cell of a CM-5 sensor chip (Biosensor) using amine coupling chemistry and the manufacturer's recommended protocols. BSA was immobilized in the same manner to another flow cell of the same chip as a control surface. Increasing concentrations of the affinity-purified rVabs were injected over both surfaces, and the binding responses were allowed to come to equilibrium. After a subtraction of the background binding (from the control surface), the equilibrium dissociation constant was derived using Scatchard analysis.

Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices) and SoftMax Pro software. Data points were averaged after a subtraction of appropriate blanks. Phage pools was
5 considered "positive" if the A_{405} of the well was > 2-fold over background.

3. Competition ELISAs

For IC_{50} determinations, microtiter plates were coated with IGF-1R and blocked as described. Phage and soluble rVabs were prepared as described above. Prior to addition of phage or soluble rVabs to the plates,
10 IGF-1 solution in PBS (1 μ g/ml) was added to duplicate wells (100 μ l/well). After incubation for 1 h at RT, the prepared phage were added to each well (100 μ l/well) without removing the IGF-1 solution. After incubation for 1 h at RT, the wells were washed and the color was developed as described above.

15 Six rVab clones bound specifically to IGF-1R. The sequences of the clones are shown in Figure 34-39.

4. Expression And Purification Of Soluble rVabs

E. coli HB2151 carrying the rVab genes on the pCANTAB5E plasmid
20 (Pharmacia) were grown in 2xYT supplemented with 100 μ g/ml ampicillin and 1% glucose at 37° C overnight and then subcultured in the absence of glucose at an OD_{600} of 0.1, and grown at 21° C until OD_{600} was 1.0. Expression was induced by the addition of IPTG to 1 mM and the cells were grown for 16 h at 30° C. The cells and culture supernatant were separated
25 by centrifugation and samples of the cell pellet and supernatant were analyzed on a 15% SDS-PAGE gel followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product. The expressed rVabs were purified from the supernatant by precipitation with ammonium sulphate
30 (which was added to 70% saturation) at 21° C, followed by centrifugation at

are agonistic with ED_{50} values of approximately 20 nM (a plot for the 43G7 antibody is shown in Figure 41). Two other rVabs, 1G2P and 39F7, have been shown to be antagonistic, with IC_{50} values of approximately 20 nM (Figure 42).

5 Microtiter wells were coated with IGF-1R as described above, with eight wells being used for each round of panning. The phage were incubated with MPBS for 30 min at RT, then 100 μ l of the phage suspension was added to each well. For the first round, the input phage titer was 8×10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately
10 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 μ l /well of MPBS. Bound phage were eluted by incubation with 100 μ l/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 4
15 cm x 4 cm plates containing 2XYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above.

20 2. Elisa Analyses Of Phage Pools

To prepare the phage pools, cells from frozen stocks were grown and phage were prepared as described above. Microtiter wells were coated and blocked as described above. The wells were coated with either IGF-1R (R&D Systems, Inc.) or with control BSA. Phage resuspended in MPBS
25 were added to duplicate wells (100 μ l/well) and incubated at RT for 1 h. The phage solution was then removed, and the wells were washed 3 times with PBS at RT. Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia) was diluted 1:3000 in MPBS and added to each well (100 μ l/well). Incubation was for 1 h at RT, followed by PBS washes as
30 described. Color was developed by addition of ABTS solution (100 μ l/well;

uncut DNA. The resulting 800 bp fragment was purified by passing DNA over QIAquick spin columns.

Phagemid pCANTAB 5E (Pharmacia) was digested with the *Sfi*I and *Nof*I restriction enzymes, which was followed by the alkaline phosphatase treatment to dephosphorylate the ends of the restriction fragments generated. The digested DNA was purified by running the digested plasmid DNA on a 1% agarose gel, followed by the DNA purification using the QIAEX II (QIAGEN) column. The vector and insert DNA were ligated overnight at 16°C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 v in a electroporation cuvette (0.1 mm gap; 0.5 ml volume, BTX, Inc.). The amount of DNA in one electroporation was 12.5 µg per 500 µl of TG1 electrocompetent cells. Immediately after the pulse, 12.5 ml of a pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added, and the transformants were grown at 37°C for 1 h. The transformants were pooled, the volume measured, and an aliquot was plated onto the 2xYT-G medium containing 100 µg/ml ampicillin (2xYT-AG) plates to determine the total number of transformants. The number of different transformants and the diversity of the library was 3×10^{10} .

The electrocompetent cell preparation, phage library amplification, library phage rescue, phage preparations and coating of microtiter plates were done as described above for the peptide library.

B. Panning for IGF-1R Binders with rVab Antibody Library

1. Panning Procedure

Panning of the antibody library was done essentially as described for the peptide library, for a total of four rounds. Of the 200 clones tested, approximately 10% bound specifically to sIGF-1R. Among these specific binders, 40% can be competed by IGF-1 for receptor binding. The clonal analysis and DNA sequencing (Figures 31-39) followed by ELISA and cell-based assays (Figures 40-46) have shown that two clones, 43G7 and M100,

of the 6-12 amino acids comprising the V_H CDR3 (indicated as "D" in Figure 30).

A total of 49 human genomic v_h genes and ten human genomic v_l genes (Figure 31) were isolated from total human genomic DNA by PCR.

- 5 The other genetic components of the library (V_h, CDR3, j_h, linker, and j_l) were derived from synthetic oligonucleotides. Assembly of these components was done using directional cloning as outlined in Figure 32 and Figure 33.

A. Ligations

- 10 The general schematic for the assembly of the rVab library ("GRABLIBTM") is provided in Figure 30. Four gene fragments (V_H, V_HCDR3/J_H/LINKER, V_L and J_L) were ligated together in the proper orientation and cloned into pCANTAB 5E (Pharmacia). Directional cloning was achieved using the *Bsr*DI restriction enzyme (Figure 32). Forty-nine
- 15 germline V_H segments and ten V_L segments encoding many of the genes from the human V_H and V_L repertoire were isolated (Figure 31) using the polymerase chain reaction. V_H CDR3 (ranging from 6 to 12 amino acids) /J_H/Linker fragments were generated by ligation of four oligonucleotides (WM 2.1, 2.2, 2.3 and 2.4) and cloning the resulting fragment into the
- 20 plasmid pUC18 previously cut with *Kpn*I and *Hind*III. The insert was then amplified using PCR and oligonucleotide primers to introduce a synthetic D-segment of 6 to 12 amino acids having a random sequence and the *Bsr*DI restriction site. The J_L gene fragments were assembled as a result of annealing of two synthetic oligonucleotides. The assembled fragments (200
- 25 ng) were used as template in a PCR amplification along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting 800 bp product was purified and concentrated with QIAquick spin columns (QIAGEN), then digested with the *Sfi*I and *Not*I restriction enzymes. Streptavidin-agarose (GibcoBRL) was added to the digestion
- 30 mixture to remove the cleaved ends of the PCR product as well as any

F. **TABLE 2:** Results of panning with the H5 secondary phage library.

Round	Total	E-Tag ⁺ , ^a		IGF-1R ⁺ , ^b	
		Number	%	Number	% Total
0	32	22	69 %	0	0 %
1	128	116	91 %	1	1 %
2	128	108	84 %	2	2 %
3	160	116	91 %	65	51 %

^aE-Tag⁺ means ELISA absorbance values >2X background. ^bIGF-1R⁺ means ELISA Absorbance >2X background. Background absorbance values are 0.05 to 0.075.

5

Each of the IGF-1R⁺ clones were sequenced, as were 15 IGF-1R⁻ clones with high E-Tag values (Absorbance >1.0). These sequences are shown in Figure 29. There is no discernible difference between binding sequences and the non-binding sequences with the exception that all of the binding sequences hold the Gly at position 6 constant. All sequences, binding and non-binding, hold the TAG stop codon constant at position 3 (the *E. coli* strain used in phage production contains the *supE44* mutation, therefore Gln replaces the TAG and it denoted in Figure 29 by Q). This suggests TAG stop codon is required for phage production and not binding.

10

15 **Example 5: Construction of the rVab Recombinant Antibody Variable Region library**

The design, expression and purification of single-chain antibodies has been reviewed (Rader and Barbas, 1997; Hoogenboom, 1997). Briefly, the variable portion of the heavy chain (V_H) is linked to the variable portion of the light chain (V_L) by a flexible peptide linker. Random combinations of V_H and V_L genes can be genetically combined to provide some of the diversity required for a library of recombinant variable region antibodies (rVabs) (Figure 30). In our library, further diversity is provided by full randomization

20

the two libraries (70%) display a peptide (i.e., are positive for E-tag), only about 6% of the clones from the A6 long (A6L) library bind to IGF-1R by phage ELISA, and none of the 24 clones tested from the A6 short (A6S) library bind to IGF-1R. This indicates that the most common outcome of random mutagenesis is the loss of IGF-1R affinity. Nevertheless, some mutants do retain their binding properties and some have improved affinities (see below).

E. Panning with the Secondary Libraries

The two secondary libraries of Example 4 were used in a panning experiment against IGF-1R. Approximately 50 clones from each four rounds of panning were analyzed in a phage ELISA to identify the clones that bind to the receptor. The positive clones were subjected to DNA sequencing and protein sequence comparison. Figure 28 provides a listing of different sequences obtained from panning with the A6S library. The results show that a variety of phage peptide sequences can bind to IGF-1R, while the consensus sequence NFYDWFV is preserved in the majority of instances.

The H5 secondary phage library was panned against IGF-1R to find H5-like peptides with higher affinities for IGF-1R

The H5 Library has a diversity of $\sim 2.6 \times 10^{10}$ clones with a phage titer of 1.0×10^{13} phage ml⁻¹. A total of three rounds of panning were performed. Table 2 summarizes the results from the three rounds of panning and shows the ELISA results for the individual clones selected from each round, the number of clones examined in each round of panning, as well as the number and percentage of E-Tag⁺ clones and IGF-1R⁺ clones.

well as versions containing high numbers of mutations per peptide in a very large library ($>10^{10}$).

Therefore, the H5 secondary mutant library was designed to contain an average of four amino acid changes (mutations) per peptide. The number of possible mutant H5 peptide sequences having four mutations is 1.0×10^{10} and is equivalent to the actual size of the secondary phage library. Sequence analysis indicates that of these peptides 30% have 3-4, 33% have 1-2 and 32 % have 5-6 mutations. There also was a small percent with 7-8 mutations and 5% clones without any mutation.

An oligonucleotide based on the DNA sequence encoding the H5 peptide was synthesized. The sequence of the oligonucleotide is:
5'-CTACAAAGACCTGTGTTAGAGTTTGGGGGTTACGTATCCGGGTTGGT
TGGCGGGGTGGTGTGCGGCCGCCAGTGTGA-3'

The underlined base positions were synthesized as mixtures of four nucleosides as follows:

A = 90% A; 3.3% C; 3.3% G; and 3.3% T

C = 3.3% C; 90% C; 3.3% G; and 3.3% T

G = 3.3% C; 3.3% C; 90% G; and 3.3% T

T = 3.3% C; 3.3% C; 3.3% G; and 90% T

Using this oligonucleotide as a template, the H5 secondary library was constructed, electroporated, amplified, and rescued essentially as described for the original peptide library. The final diversity of this secondary library was $\sim 10^{10}$.

D. Characterization of Libraries

Forty-eight randomly picked clones from each of the secondary libraries (Round 0, before panning) were rescued and the phage was assayed in an ELISA for binding to the anti-E-tag mAb, as well as for binding to IGF-1R (E-tag is used as an indicator of expression of displayed peptides on phage surfaces). The results showed that although most of the clones in

pCANTAB5E vector as described for the original peptide library. Over 10^{10} different clones were obtained in the final library.

B. Phage Library A6S

While the consensus sequence NFYDWFV was kept constant in the A6S library, the flanking regions were randomized in the A6S library as shown in Figure 26A. The codons in the random region were of the NNK type to reduce the frequency of stop codons (N = A, C, G, or T; K = G or T). The sequence of the synthetic oligonucleotide made is given in Figure 26B. This synthetic oligonucleotide was then used as a template in a PCR reaction. The product of this PCR reaction was then purified, cut with *Sfi* I and *Not* I restriction enzymes and cloned into the pCANTAB5E vector as described for the original peptide library. Over 10^9 different clones were obtained in the final library.

C. Secondary Phage Library Based on Clone H5

Peptide H5 (LCQRLGVGWPGWLSGWCA) was identified in an independent experiment as a binder to the rat growth hormone binding protein. This peptide and four other H5-like peptides, including 2C3-60 (Figure 27), were found in cell culture experiments to possess agonistic activity toward IGF-1R⁺ cells, but not against IGF-1R⁻ cells. Further, subsequent *in vitro* experiments showed that the H5-like peptides are not competed by IGF. This suggests that these peptides recognize a second allosteric site on IGF-1R. BIAcore analysis showed that binding of the 2C3-60 peptide to IGF-1R is ~ 20 μ M. Subsequently, a phage library of mutants of the H5 sequence was constructed and used for panning against IGF-1R. Gene synthesis to introduce mutations and phage display were used to construct an H5 secondary library. In this method a library of doped oligonucleotides carrying several mutations in any single DNA molecule is used to obtain a pool of mutant genes which are phage displayed. This method allowed the encoding of both the original H5 peptide as control as

Exempl 4: Construction of Secondary Phage Libraries

Two phage libraries were designed on the basis of the sequences of the Class II binders known to possess agonistic properties in cell-based assays. The goal was to bring the affinity into a range that would allow the peptide to be used in a receptor binding assay and tested in a cell based assay for activity. Among several available mutagenesis methods, we chose one based on gene synthesis and phage display. In this method a library of doped oligonucleotides carrying several mutations in any single DNA molecule is used to obtain a pool of mutant genes, the expression products of which are phage displayed.

A. Phage Library A6L

The approach used was the doped synthesis of the oligonucleotide encoding the sequence of the peptide. The sequence encoding the peptide and the sequence of the synthetic oligonucleotide made are shown in Figures 25A-25B. The amino acid residues belonging to the consensus sequence were kept constant and were not mutated. The ratio of nucleosides in each condensation was chosen to provide an average of 6 nucleotide sequence changes at the DNA level and 4-5 mutations at the amino acid level over the length of the peptides. The regions corresponding to the FLAG, *Sfi*I and *Nof*I sites were not mutated.

The DNA sequence encoding the A6 peptide was optimized for *E. coli* codon usage by replacing a total of 24 nucleotides as shown in Figure 25A. The TAG stop codons (suppressed in the TG1 *E. coli* strain used) were replaced with CAG (glutamine). Then, the oligonucleotide sequence was designed to include doped nucleosides at positions corresponding to the coding region for the A6 peptide, except for the consensus NFYDWFV (Figure 25A). This synthetic oligonucleotide (Figure 25B) was then used as a template in a PCR reaction. The product of this PCR reaction was then purified, cut with *Sfi*I and *Nof*I restriction enzymes and cloned into the

F-Y-x-x-L-s-x-L, and are shown to be antagonistic in cell-based assays (Figure 22). Class II peptides contain the consensus N-F-Y-D-W-F-V, and are shown to be agonistic in cell-based assays (Figure 23). Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature IGF-1.

Example 3: Assays with Synthetic Peptides

Four synthetic peptides, 5.1, 5.2, 5.3 and 5.4 (Figure 21) were made to study the properties of the artificial peptide ligands from phage display.

Synthetic peptides were obtained from a commercial supplier (Anaspec). The peptides were supplied greater than 90% pure by HPLC. The molecular weights of the peptides as determined by mass spectroscopy agreed with the expected values.

IGF-1R (100 µg/ml) was immobilized onto one flow-cell of a CM-5 sensor chip (Biosensor) using amine coupling chemistry and the manufacturer's recommended protocol. An unrelated IgG was immobilized in the same manner to another flow cell of the same chip as a control surface. Increasing concentrations of synthetic peptide were injected over both surfaces, and the binding responses were allowed to come to equilibrium. After subtraction of background binding from the control surface, the results were used to derive an equilibrium dissociation constant using Scatchard analysis (Figure 24A).

In another experiment, IGF-1R (100 µg/ml) was immobilized onto a CM-5 sensor chip as described above, and an unrelated IgG was immobilized in the same manner to another flow cell of the same chip. IGF-1 alone, peptide 5.1 alone (corresponding to the B6 phage clone), or different mixes of the two, were injected over the derivatized chip surfaces. The results shown in Figure 24B indicate that the 5.1 peptide inhibits the binding of IGF-1, and the inhibition is increased by increasing amounts of the peptide. The results support the idea of an overlap of the peptide 5.1 binding site and the IGF-1 binding site on IGF-1R.

was developed by addition of ABTS solution (100 μ l/well; Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices Corp., Sunnyvale CA) and SoftMax Pro software. Data points were averaged after subtraction of appropriate blanks. A clone was considered "positive" if the A_{405} of the well was \geq 2-fold over background.

For IC_{50} determinations in a competitive ELISA, microtiter plates were coated with IGF-1R and blocked as described. Phage were prepared as described. Prior to addition of phage to plates, the peptide or recombinant variable antibody or fragment ("rVab"), or an appropriate control, was diluted in PBS and added to duplicate wells (100 μ l/well). After incubation for 1 h at RT, the prepared phage were added to each well (100 μ l/well) without removing the peptide or rVab solution. After incubation for 1 h at RT, the wells were washed and the color developed as described above.

The clones were next analyzed for binding to the receptor's active site (Figures 20A and 20B). Competitions of phage binding were done with the cognate ligand (i.e., IGF-1). All four phage clones tested, B6, F6, C6 and E5, bound to same site as IGF-1 since the binding of the clones to the immobilized IGF-1R could be inhibited with IGF-1.

To determine the rank order for phage peptides, the human IGF-1R (25 g/ml) was immobilized onto a CM-5 (BIAcore) sensor chip using amino coupling chemistry and the manufacturer's recommended protocol. The final surface density was 1000 RU. A monoclonal antibody was immobilized onto another flow cell as a control surface. Phage were directly injected (30-100 μ l) with a buffer flow rate of 1 μ l/min. Background binding to the control surface was subtracted prior to further analysis.

C. Phage Sequence Analysis

Sequence analysis of several clones shows that there are two distinct populations, designated as Class I (Formula motif 2) and Class II (Formula motif 1; Figure 21). Several of these have been chemically synthesized for subsequent testing. Class I peptides contain the consensus sequence D-x-

Eight wells were used for each round of panning. The phage were incubated with MPBS for 30 min at RT, then 100 μ l was added to each well. For the first round, the input phage titer was 4×10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 μ l/well of MPBS. Bound phage were eluted by incubation with 100 μ l/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A minimum of 72 clones were picked at random from the second, third, and fourth rounds of panning and screened for binding activity. DNA sequencing of the clones revealed the abundance of sequences as summarized in Figure 18. Some of the clones (Figure 19) were frameshifted, that is, the relevant peptide sequence was encoded not in the FLAG frame, but in either frame + 1 or - 1.

B. ELISA Analyses of Phage

For phage pools, cells from frozen stocks were grown and phage were prepared as described above. For analysis of individual clones, colonies were picked and phage prepared as described above. Subsequent steps are the same for pooled and clonal phage. Microtiter wells were coated and blocked as described above. Wells were coated with either IGF-1R or a control IgG mAb. Phage resuspended in MPBS were added to duplicate wells (100 μ l/well) and incubated at RT for 1 h. The phage solution was then removed, and the wells were washed 3 times with PBS at RT. Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia) was diluted 1:3000 in MPBS and added to each well (100 μ l/well). Incubation was for 1 h at RT, followed by PBS washes as described. Color

- OD₆₀₀ = 0.5 in 2xYT-AG at 30°C with shaking (250 rpm). Helper phage (M13K07) was then added (multiplicity of infection (MOI) = 15), and the cells were incubated for 30 min at 37° C without shaking, followed by 30 min at 37°C with shaking (250 rpm). Following infection, cells were pelleted and
- 5 the supernatant containing the helper phage was discarded. The cell pellet was resuspended in the initial culture volume of 2xYT-A (no glucose) containing 50 mg/ml kanamycin and grown overnight at 30°C with shaking (250 rpm). The cells from the overnight culture were pelleted at 3000 x g for 30 min at 4°C and the supernatant containing the phage was recovered.
- 10 The solution was adjusted to 4% PEG, 500 mM NaCl and chilled on ice for 1 h. The precipitated phage were pelleted by centrifugation at 10,000 x g for 30 min, then resuspended in phosphate-buffered saline (1/100 of the initial culture volume) and passed through a 0.45 µm filter. The phage were
- 15 titrated by infecting TG1 cells. The phage titer for the 40mer peptide library was 4×10^{13} cfu/ml. The phage titer for the 20mer library was 3×10^3 .

To amplify the library, the transformants were inoculated into 4 l of 2xYT-AG medium and allowed to grow until the OD₆₀₀ increased approximately 400 times. The cells were pelleted by centrifugation at 3000 x g for 20 min, then resuspended in 40 ml 2xYT-AG to which glycerol was

20 added to a final concentration of 8%. The library was stored at -80°C.

Example 2:

A. Panning IGF-1R

- A standard method was used to coat and block all microtiter plates. The soluble IGF-1R ("sIGF-1R") was diluted to 1 mg/ml in 50 mM sodium
- 25 carbonate buffer, pH 9.5. One hundred microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with MPBS (PBS buffer pH 7.5 containing 2% Carnation® non-fat dry milk) at room temperature (RT) for 1 h.

et al., 1988) was incorporated into the library as an immunoaffinity tag as shown in Figure 16.

Another phage library expressing 20mer peptides, was constructed according to a similar procedure. The diversity of the library is 1.1×10^{11}
5 different clones.

B. Preparation of Electrocompetent Cells

To prepare electrocompetent cells, an overnight culture of *E. coli* TG1 cells (*F'*_{traD36} *lacI*^r $\Delta(lacZ)$ M15 *proAB*) / *supE* $\Delta(hsdM-mcrB)$ ₅ *r*_k⁻*m*_k⁻ *McrB*⁻ *thi* $\Delta(lac-proAB)$ was diluted to an OD₆₀₀ = 0.05-0.1 in 500 ml 2xYT, then
10 grown at 37°C in 4 liter Ehrlenmyer flasks to an OD₆₀₀ = 0.5-0.6. The culture was poured into pre-chilled centrifuge bottles and incubated on ice for 30 min prior to centrifugation at 2000 x g for 30 min (2°C). The supernatant was poured off and the cell pellet was resuspended in a total of 400 ml of ice cold sterile distilled water. The process of centrifugation and resuspension
15 was repeated 2 times. After the last centrifugation, the pellet was resuspended in a total of 25 ml of ice cold water containing 10% glycerol. The cell suspension was transferred to pre-chilled 35 ml centrifuge bottles, and was then pelleted at 2000 x g for 10 min at 4°C. The cells were then suspended in 0.3 ml of the same 10% glycerol solution, aliquotted into
20 smaller tubes, and snap-frozen on dry ice. The aliquots were stored at -80°C.

To amplify the library, the transformants were inoculated into 4 l of 2xYT-AG medium and allowed to grow until the A₆₀₀ increased approximately 400 times. The cells were pelleted by centrifugation at 3000
25 x g for 20 min, then resuspended in 40 ml 2xYT-AG to which glycerol was added to a final concentration of 8%. The library was stored at -80°C.

C. Phage Rescue

This process was carried out using the standard phage preparation protocol with the following changes. Five individual recombinant cell
30 libraries, with a total diversity of 1.6×10^{10} , were combined and grown to

Example 1

A. Construction of Phage Library for Identifying IGF-1R and IR Binding Ligands

The schematic for the peptide library "RAPIDLIB™" on filamentous phage is shown in Figure 16. DNA fragments coding for peptides containing 40 random amino acids were generated in the following manner. A 145 base oligonucleotide was synthesized to contain the sequence (NNK)₄₀, where N = A, C, T, or G, and K = G or T. This oligonucleotide was used as the template in a PCR amplification along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting 190 bp product was purified and concentrated with QIAquick spin columns (QIAGEN, Inc. Valencia, CA), then digested with *Sfi*I and *Not*I. Streptavidin-agarose (GibcoBRL Life Technologies, Inc., Rockville, MD) was added to the digestion mixture to remove the cleaved ends of the PCR product as well as any uncut DNA. The resulting 150 bp fragment was again purified over QIAquick spin columns. The phagemid pCANTAB5E (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) was digested with *Sfi*I and *Not*I, followed by phosphatase treatment. The digested DNA was purified using a 1% agarose gel followed by QIAEX II (QIAGEN). The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick spin columns (QIAGEN). Electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 12.5 µg of DNA and 500 µl of TG1 electrocompetent cells (see below). Immediately after the pulse, 12.5 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were pooled, the volume measured, and an aliquot was plated onto 2xYT-G containing 100 g/ml ampicillin (2xYT-AG) plates to determine the total number of transformants.

Sequence analysis of randomly selected clones indicated that 54% of all clones are in-frame (Mandecki *et al.*, 1997). The FLAG sequence (Hopp

cells at various stages of development, including the embryonic and fetal stages, so as to effect gene therapy at early stages of development.

The described constructs may be administered in the form of a pharmaceutical preparation or composition containing a pharmaceutically acceptable carrier and a physiological excipient, in which preparation the vector may be a viral vector construct, or the like, to target the cells, tissues, or organs of the recipient organism of interest, including human and non-human mammals. The composition may be formed by dispersing the components in a suitable pharmaceutically acceptable liquid or solution such as sterile physiological saline or other injectable aqueous liquids. The amounts of the components to be used in such compositions may be routinely determined by those having skill in the art. The compositions may be administered by parenteral routes of injection, including subcutaneous, intravenous, intramuscular, and intrasternal.

The following non-limiting examples illustrate various aspects and embodiments of the invention and should not be contrived as limiting the scope of the invention.

VI. EXAMPLES

The following materials were used in the examples described below.

Soluble IGF-1R was obtained from R&D Systems (Cat. # 391-GR/CF). Insulin receptor was prepared according to Bass *et al.*, 1996. The insulin is either from Sigma (Cat. # I-0259) or Boehringer. The IGF-1 is from PeproTech (Cat. # 100-11). All synthetic peptides were synthesized by Novo Nordisk, AnaSpec, Inc. (San Jose, CA), PeptioGenics (Livermore, CA), or Research Genetics (Huntsville, AL) at >80% purity. The Maxisorb Plates are from Nunc via Fisher (Cat. # 12565347). The HRP/Anti-M13 Conjugate is from Pharmacia (Cat. # 27-9421-01). The ABTS solution is from BioF/X (Cat. # ABTS-0100-04).

plasmid) to a protein ligand via polylysine. The appropriate or suitable ligands are selected on the basis of the presence of the corresponding ligand receptors on the cell surface of the target cell or tissue type. These ligand-DNA conjugates can be injected directly into the blood, if desired, and
5 are directed to the target tissue where receptor binding and DNA-protein complex internalization occur. Co-infection with adenovirus to disrupt endosome function can be used to overcome the problem of intracellular destruction of DNA.

An approach that combines biological and physical gene transfer
10 methods utilizes plasmid DNA of any size combined with a polylysine-conjugated antibody specifically reactive with the adenovirus hexon protein. The resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector allows efficient binding to the cell, internalization, and degradation of the endosome before
15 the coupled DNA can be damaged.

Many types of cells and cell lines (e.g. primary cell lines or established cell lines) and tissues are capable of being stably transfected by or receiving the constructs of the invention. Examples of cells that may be used include, but are not limited to, stem cells, B lymphocytes, T
20 lymphocytes, macrophages, other white blood lymphocytes (e.g. myelocytes, macrophages, monocytes), immune system cells of different developmental stages, erythroid lineage cells, pancreatic cells, lung cells, muscle cells, liver cells, fat cells, neuronal cells, glial cells, other brain cells, transformed cells of various cell lineages corresponding to normal cell
25 counterparts (e.g. K562, HEL, HL60, and MEL cells), and established or otherwise transformed cells lines derived from all of the foregoing. In addition, the constructs of the present invention may be transferred by various means directly into tissues, where they would stably integrate into the cells comprising the tissues. Further, the constructs containing the DNA
30 sequences of the peptides of the invention can be introduced into primary

unit containing the gene to be introduced into a specific cell for replacement gene therapy; a selectable gene as described below; and a 3' LTR which contains a deletion in the viral enhancer region, or deletions in both the viral enhancer and promoter regions. The selectable gene may or may not have
5 a 5' promoter that is active in the packaging cell line, as well as in the transfected cell.

The recombinant retroviral vector DNA can be transfected into the amphotrophic packaging cell line Ψ -AM (see Cone and Mulligan, 1984) or other packaging cell lines which are capable of producing high titer stocks of
10 helper-free recombinant retroviruses. After transfection, the packaging cell line is selected for resistance to G418, present at appropriate concentration in the growth medium. Adenoviral vectors (e.g. DNA virus vectors), particularly replication-defective adenovirus vectors, or adeno-associated vectors, have been described in the art (Kochanek *et al.*, 1996; Ascadi *et al.*,
15 1994; Ali *et al.*, 1994).

Nonviral gene transfer methods known in the art include chemical techniques, such as calcium phosphate co-precipitation, direct DNA uptake and receptor-mediated DNA transfer, and mechanical means, such as microinjection and membrane fusion-mediated liposomal transfer. In
20 addition, viral-mediated gene transfer can be combined with direct *in vivo* gene transfer using liposomes, thereby allowing the delivery of the viral vectors to tumor cells, for example, and not to surrounding non-proliferating cells. A description of various liposomes which are stated as being useful for transferring DNA or RNA into cells is present in United States Patents
25 5,283,185 and 5,795,587. The retroviral vector producer cell line can also be injected directly into specific cell types, e.g., tumors, to provide a continuous source of viral particles, such as has been approved for use in patients afflicted with inoperable brain tumors.

Receptor-mediated gene transfer methods allow targeting of the DNA
30 in the construct directly to particular tissues. This is accomplished by the conjugation of DNA (frequently in the form of a covalently closed supercoiled

quantities, as well as routes of administration, used are determined on an individual basis, and correspond to the amounts used in similar types of applications or indications known to those of skill in the art.

The constructs as described herein may also be used in gene transfer and gene therapy methods to allow the expression of one or more amino acid sequences of the present invention. Using the amino acid sequences of the present invention for gene therapy may provide an alternative method of treating diabetes which does not rely on the administration or expression of insulin. Expressing insulin for use in gene therapy requires the expression of a precursor product, which must then undergo processing including cleavage and disulfide bond formation to form the active product. The amino acid sequences of this invention, which possess activity, are relatively small, and thus do not require the complex processing steps to become active. Accordingly, these sequences provide a more suitable product for gene therapy.

Gene transfer systems known in the art may be useful in the practice of the invention. Both viral and non-viral methods are suitable. Examples of such transfer systems include, but are not limited to, delivery via liposomes or via viruses, such as adeno-associated or vaccinia virus. Numerous viruses have been used as gene transfer vectors, including papovaviruses (e.g., SV40, adenovirus, vaccinia virus, adeno-associated virus, herpes viruses, including HSV and EBV, and retroviruses of avian, murine, and human origin). As is appreciated by those in the art, most human gene therapy protocols have been based on disabled murine retroviruses. Recombinant retroviral DNA can also be employed with amphotrophic packaging cell lines capable of producing high titer stocks of helper-free recombinant retroviruses (e.g., Cone and Mulligan, 1984).

A recombinant retroviral vector may contain the following parts: an intact 5' LTR from an appropriate retrovirus, such as MMTV, followed by DNA containing the retroviral packaging signal sequence; the insulator element placed between an enhancer and the promoter of a transcription

useful for the treatment or prevention of diabetic retinopathy. Recent reports have shown that a previously identified IGF-1R antagonist can suppress retinal neovascularization, which causes diabetic retinopathy (Smith *et al.*, 1999).

5 IGF-1R agonist amino acid sequences provided by this invention are useful for development as treatments for neurological disorders, including stroke and diabetic neuropathy. Reports of several different groups implicate IGF-1R in the reduction of global brain ischemia, and support the use of IGF-1 for the treatment of diabetic neuropathy (reviewed in Auer *et al.*, 1998; Apfel, 1999).

J. Methods of Administration

The amino acid sequences of this invention may be administered as pharmaceutical compositions comprising standard carriers known in the art for delivering proteins and peptides and by gene therapy. Due to the labile
15 nature of the amino acid sequences parenteral administration is preferred. Preferred modes of administration include aerosols for nasal or bronchial absorption; suspensions for intravenous, intramuscular, intrasternal or subcutaneous, injection; and compounds for oral administration. Other modes of administration and examples of suitable formulative components
20 for use with this embodiment are discussed below. Other modes of administration include intranasal, intrathecal, intracutaneous, percutaneous, enteral, and sublingual. For injectable administration, the composition is in sterile solution or suspension or may be emulsified in pharmaceutically- and physiologically-acceptable aqueous or oleaginous vehicles, which may
25 contain preservatives, stabilizers, and material for rendering the solution or suspension isotonic with body fluids (i.e. blood) of the recipient. Excipients suitable for use are water, phosphate buffered saline, pH 7.4, 0.15 M aqueous sodium chloride solution, dextrose, glycerol, dilute ethanol, and the like, and mixtures thereof. Illustrative stabilizers are polyethylene glycol, ~~and~~
30 proteins, saccharides, amino acids, inorganic acids, and organic acids, which may be used either on their own or as admixtures. The amounts or

I. Use of the Peptides Provided by this Invention

The IR and IGF-1R agonist and antagonist peptides provided by this invention are useful as potential therapeutics in pharmaceutical compositions, lead compounds for identifying other more potent or selective therapeutics, assay reagents for identifying other useful ligands by, for example, competition screening assays, and as research tools for further analysis of IR and IGF-1R. In particular, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which include members that bind to Site 1 and/or Site 2 of IR or IGF-1R. Such libraries can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

IR agonist amino acid sequences provided by this invention are useful as insulin analogs and may therefore be developed as treatments for diabetes or other diseases associated with a decreased response or production of insulin. For use as an insulin supplement or replacement, preferred amino acid sequence are: FHENFYDWFVRQVSK (D117, H2C), DYKDFYDAIQLVRSARAGGTRDKK (D118, 20E2), KDRAFYNGLRDLVGAVYGAWDKK (D119, 20C11), DYKDLCQSWGVRIGWLAGLCPKK (D116, JBA5), DYKDVTFSTSAVFHENFYDWFVRQVSKK (D113, H2), and GRVDWLQRNANFYDWFVAELG (S175). More preferred IR agonists are: FHENFYDWFVRQVSK (D117, H2C) and GRVDWLQRNANFYDWFVAELG (S175). Most preferred is GRVDWLQRNANFYDWFVAELG (S175). Preferred dimer sequences are represented by S170, S171, S172, S232, S300 sequences (see Table 15).

IGF-1R antagonist amino acid sequences provided by this invention are useful as treatments for cancers, including, but not limited to, breast and prostate cancers. Human and breast cancers are responsible for over 40,000 deaths per year, as present treatments such as surgery, chemotherapy, radiation therapy, and immunotherapy show limited success. The IGF-1R antagonist amino acid sequences disclosed herein are also

pharmacophore, rather than the bonding between atoms), and other techniques can be used in this modeling process.

In a variant of this approach, the three dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, does not degrade *in vivo*, and retains the biological activity of the lead compound. The mimetics found are then screened to ascertain the extent they exhibit the target property, or to what extent they inhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

This invention provides specific IR and IGF-1R amino acid sequences which function as either agonists or antagonists at IR and/or IGF-1R. Examples of phage display libraries suitable for use in this invention include one such library containing randomized 40 amino acid peptides (RAPIDLIB™, Figure 16), another library containing rVab derived from human genomic antibody DNA (GRABLIB™, Figure 30). Details of the construction and analyses of these libraries, as well as the basic procedures for biopanning and selection of binders, have been described elsewhere (WO 96/04557; Mandecki *et al.*, 1997; Ravera *et al.*, 1998; Scott and Smith, 1990); Grihalde *et al.*, 1995; Chen *et al.*, 1996; Kay *et al.*, 1993, Carcamo *et al.*, 1998, all of which are incorporated herein by reference). Another phage display library suitable for use with this invention is available commercially from New England Biolabs (Ph.D. C7C Disulfide Constrained Peptide Library). Additional sequences may be obtained in accordance with the procedures described herein.

In these assays, soluble IR is coated on the surface of microtiter wells, blocked by a solution of 0.5% BSA and 2% non-fat milk in PBS, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon
5 addition of the acidic enhancement solution, the bound europium is released as free Eu^{3+} which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IR:bP bound saEu is then converted into its highly fluorescent state and detected by a detector such as Wallac Victor II (EG&G Wallac, Inc.)

10 The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g. peptides are generally unsuitable
15 active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic
20 from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide (e.g. by substituting each residue in turn). These parts or residues constituting the
25 active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties (e.g. stereochemistry, bonding, size and/or charge), using data from a range of sources (e.g. spectroscopic techniques, X-ray diffraction data and NMR). Computational analysis,
30 similarity mapping (which models the charge and/or volume of a

H. Screening Assays

In another embodiment of this invention, screening assays to identify pharmacologically active ligands at IR and/or IGF-1R are provided. The screening assays provided in accordance with this invention are based on those disclosed in International application WO 96/04557 which is incorporated herein in its entirety. Briefly, WO 96/04557 discloses the use of reporter peptides which bind to active sites on targets and possess agonist or antagonist activity at the target. These reporters are identified from recombinant libraries and are either peptides with random amino acid sequences or variable antibody regions with at least one CDR region which has been randomized (rVab). The reporter peptides may be expressed in cell recombinant expression systems, such as for example in *E. coli*, or by phage display. See WO 96/04557 and Kay *et al.* (1996), both of which are incorporated herein by reference. The reporters identified from the libraries may then be used in accordance with this invention either as therapeutics themselves, or in competition binding assays to screen for other molecules, preferably small, active molecules, which possess similar properties to the reporters and may be developed as drug candidates to provide agonist or antagonist activity. Preferably, these small organic molecules are orally active.

The basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for insulin may be as follows: occupation of the active site of IR is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IR (i.e., IR:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993). The assay can use a single-chain antibody or a biotinylated peptide. Furthermore, both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IR by insulin.

example, pRSET vectors (Invitrogen Corp., San Diego, CA). The expressed, tagged peptides can then be purified from a crude lysate of the cell-free translation system or host cell by chromatography on an appropriate solid-phase matrix.

5 Methods for directly purifying peptides from natural sources such as cellular or extracellular lysates are well known in the art (see Harris and Angal, 1989). Such methods include, without limitation, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), preparative disc-gel electrophoresis, isoelectric focusing, high-performance liquid
10 chromatography (HPLC), reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution, and combinations thereof. Naturally occurring peptides can be purified from many possible sources, for example, plasma, body tissues, or body fluid lysates derived from human or animal, including mammalian, bird, fish, and insect sources.

15 Antibody-based methods may also be used to purify naturally occurring or recombinantly produced peptides. Antibodies that recognize these peptides or fragments derived therefrom can be produced and isolated. The peptide can then be purified from a crude lysate by chromatography on an antibody-conjugated solid-phase matrix (see Harlow
20 and Lane, 1998).

2. Chemical Synthesis Of Peptides

 Alternately, peptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or
25 classical solution synthesis. The polypeptides are preferably prepared by solid-phase peptide synthesis; for example, as described by Merrifield (1965; 1997). In addition, recombinant and synthetic methods of polypeptide production can be combined to produce semi-synthetic polypeptides.

carried out using established methods. DNA sequences can be optimized, if desired, for more efficient expression in a given host organism. For example, codons can be altered to conform to the preferred codon usage in a given host cell or cell-free translation system using techniques routinely
5 practiced in the art.

Suitable cell-free systems for expressing recombinant peptides include, for example, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *Escherichia coli* (*E. coli*) S30 extract, and coupled transcription/translation systems (Promega Corp., Madison,
10 WI). Such systems allow expression of recombinant polypeptides upon the addition of cloning vectors, DNA fragments, or RNA sequences containing coding regions and appropriate promoter elements.

Host cells for cloning vectors include bacterial, archebacterial, fungal, plant, insect and animal cells, especially mammalian cells. Of particular
15 interest are *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*, SF9, C129, 293, NIH 3T3, CHO, COS, and HeLa cells. These cells can be transformed, transfected, or transduced, as appropriate, by any suitable method including electroporation, CaCl_2 -, LiCl -, LiAc/PEG -,
20 spheroplasting-, Ca-Phosphate , DEAE-dextran, liposome-mediated DNA uptake, injection, microinjection, microprojectile bombardment, or other established methods.

For some purposes, it may be preferable to produce peptides in a recombinant system in which they carry additional sequence tags to
25 facilitate purification. Non-limiting examples of tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS), GLU-GLU, and DYKDDDDK (FLAG®) epitope tags. Epitope tags can be added to peptides by a number of established methods. DNA sequences of epitope tags can be inserted into peptide coding sequences as oligonucleotides or through primers used
30 in PCR amplification. As an alternative, peptide coding sequences can be cloned into specific vectors that create fusions with epitope tags; for

Other combinations of peptides are within the scope of this invention and may be determined as demonstrated in the examples described herein.

Regarding preparation of a Site 1 agonist comprising two D117 (H2C) peptides, a linker of only 3 amino acids (12 Å) provided a ligand of greater
5 affinity for Site 1 of IR than a corresponding ligand prepared with a 9 amino acid (36 Å) linking region. Figure 17.

Notably, several fusion peptides show IR agonist activity as determined by an IR autophosphorylation assay (see Example 20). Figure 74. In particular, fusion peptides 439, 436, 449, and 463 show significant IR
10 agonist activity (Figure 74).

G. Peptide Synthetic Techniques

Many conventional techniques in molecular biology, protein biochemistry, and immunology may be used to produce the amino acid sequences for use with this invention.

15 1. Recombinant Synthesis

To obtain recombinant peptides, the corresponding DNA sequences may be cloned into any suitable vectors for expression in intact host cells or in cell-free translation systems by methods well known in the art (see
20 Sambrook *et al.*, 1989). The particular choice of the vector, host, or translation system is not critical to the practice of the invention.

Cloning vectors for the expression of recombinant peptides include, but are not limited to, pUC, pBluescript (Stratagene, La Jolla, CA), pET (Novagen, Inc., Madison, WI), pMAL (New England Biolabs, Beverly, MA), or pREP (Invitrogen Corp., San Diego, CA) vectors. Vectors can contain
25 one or more replication and inheritance systems for cloning or expression, one or more markers for selection in the host (e.g. antibiotic resistance), and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids, etc. Ligation of the coding sequences to transcriptional
30 regulatory elements and/or to other amino acid coding sequences can be

TABLE 1

Fusion	Seq.	Action	Site	Fusion Concentration	MW (kDa)	K _d (HIR)
426	D8	N	2	0.76	52.2	1.4 x 10 ⁻⁶
429	D8-6aa-D8	N-N	2-2	3.2	55.3	1.3 x 10 ⁻⁶
430	H2C-6aa-RB6	A-	1-1	0.17	54.5	2.1 x 10 ⁻⁶
431	H2C-6aa-F8	A-N	1-2	3.3	54.8	4.7 x 10 ⁻⁶
432	H2C-12aa-F8	A-N	1-2	2.9	55.5	3.5 x 10 ⁻⁶
433	H2C-9aa-F8	A-N	1-2	2.8	55.2	2.1 x 10 ⁻⁶
434	G3-12aa-G3	N-N	1-1	0.01	56	3.2 x 10 ⁻⁶
436	H2C-9aa-H2C	A	1-1	1.1	54.2	4.1 x 10 ⁻⁷
437	H2C	N-N	1	0.3	51.5	8.3 x 10 ⁻⁶
427	G3-6aa-G3	N-N	1-1	0.02	55.3	3.3 x 10 ⁻⁶
435	H2C-3-H2C-3-H2C	A-A-A	1-1-1	2.1	55.5	2.0 x 10 ⁻⁶
439	H2C-6aa-H2C	A-A	1-1	1.4	53.9	5.5 x 10 ⁻⁷
449	H2C-12aa-H2C		1-1	1.5	51.8	6.2 x 10 ⁻⁷
452	G3		1	0.15	48.8	7.8 x 10 ⁻⁷
463	H2C-3aa-H2C	A-A	1-1	1.8	50.1	9.6 x 10 ⁻⁷
464	LF-H2C		1	0.045	48.4	3.9 x 10 ⁻⁶
446	LF-F8		2	1.9	49.1	7.7 x 10 ⁻⁷
459	SF-RB6			0.069	48.1	7.7 x 10 ⁻⁶
MBP*	lacZ			5.1	50	> 1 x 10 ⁻⁵

*MBP (negative control for the fusions) is fused to a small fragment of beta-galactosidase (lacZ).

N = Antagonist

A = Agonist

LF = Long FLAG epitope (DYKDDDDK)

SF = Short FLAG epitope (DYKD)

Additional binding data for the fusion peptides are shown below:

Fusion	Highest conc. tested (μM)	K _d (HIR) μM
431-	0.2	0.033
431+	0.2	0.0074
432-	0.2	0.02
432+	0.2	0.0038
433-	0.2	0.03
433+	0.2	0.004

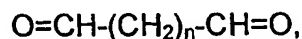
The concentrations of these fusions vary depending on the expression quality.

- 5 There are 2 sets of each fusion: uncleaved (-) and cleaved with factor Xa (+). The fusion proteins are in Tris buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 50 mM maltose, pH 7.5) and the cleaved fusions (+) are in the same Tris buffer (500 μl) + 12 μg Factor Xa. (Source of Factor Xa: New England Biolabs).

construct illustrated above, G and S are glycine and serine residues, which make up the linker sequence.

In addition to producing the dimer peptides by recombinant protein expression, dimers may also be produced by peptide synthesis whereby a synthetic technique such as Merrifield synthesis (Merrifield, 1997), may be used to construct the entire peptide.

Other methods of constructing dimers include introducing a linker molecule which activates the terminal end of a peptide so that it can covalently bind to a second peptide. Examples of such linkers include diaminopropionic acid activated with an oxyamino function. A preferred linker is a dialdehyde having the formula



Wherein n is 2 to 6, but is preferably 6 to produce a linker of about 25 to 30 angstroms in length. Linkers may be used to link dimers either to the carboxyl terminal or the amino terminal.

2. Characterization Of Specific Dimers

Specific dimers which bind with high affinity to Site 1, Site 2, or both Site 1 and Site 2 of the insulin receptor are shown in Table 1. Although agonist activity has been observed for the Site 1-Site 1 dimers, the Site 1-Site 2 dimers may also possess desirable properties.

the construct. Such sequences optionally may be subsequently removed to produce the mature binding ligand. Recombinant expression also provides means for producing large quantities of ligand. In addition, recombinant expression may be used to express different combinations of amino acid sequences and to vary the orientation of their combination, i.e., amino to carboxyl terminal orientation.

Whether produced by recombinant gene expression or by conventional chemical linkage technology, the various amino acid sequences may be coupled through linkers of various lengths. Where linked sequences are expressed recombinantly, and based on an average amino acid length of about 4 angstroms, the linkers for connecting the two amino acid sequences would typically range from about 3 to about 12 amino acids corresponding to from about 12 to about 48 Å. Accordingly, the preferred distance between binding sequences is from about 2 to about 50 Å. More preferred is 4 to about 40. The degree of flexibility of the linker between the amino acid sequences may be modulated by the choice of amino acids used to construct the linker. The combination of glycine and serine is useful for producing a flexible, relatively unrestrictive linker. A more rigid linker may be constructed by using amino acids with more complex side chains within the linkage sequence.

In a preferred embodiment shown below (Figure 16)

MBP-FLAG-PEPTIDE-(G,S)_n-PEPTIDE-E-TAG

a fusion construct producing a dipeptide comprises a maltose binding protein amino acid sequence (MBP) or similar sequence useful for enabling the affinity chromatography purification of the expressed peptide sequences. This purification facilitating sequence may then be attached to a flag sequence to provide a cleavage site to remove the initial sequence. The peptide dimer then follows which includes the intervening linker and a tag sequence may be included at the carboxyl terminal portion to facilitate identification/purification of the expression of peptide. In the representative

different subunits of a receptor, the amino acid sequences of the ligand for binding to the receptors may be the same or different, provided that if different amino acid sequences are used, they both bind to the same site.

5 Multivalent ligands may be prepared by either expressing amino acid sequences which bind to the individual sites separately and then covalently linking them together, or by expressing the multivalent ligand as a single amino acid sequence which comprises within it the combination of specific amino acid sequences for binding.

10 Various combinations of amino acid sequences may be combined to produce multivalent ligands having specific desirable properties. Thus, agonists may be combined with agonists, antagonists combined with antagonists, and agonists combined with antagonists. Combining amino acid sequences which bind to the same site to form a multivalent ligand may be useful to produce molecules which are capable of cross-linking together
15 multiple receptor units. Multivalent ligands may also be constructed to combine amino acid sequences which bind to different sites (Figure 15).

In view of the discovery disclosed herein of monomers having agonist properties at IR or IGF-1R, preparation of multivalent ligands may be useful to prepare ligands having more desirable pharmacokinetic properties due to
20 the presence of multiple bind sites on a single molecule. In addition, combining amino acid sequences which bind to different sites with different affinities provides a means for modulating the overall potency and affinity of the ligand for IR or IGF-1R.

1. Construction of Hybrids

25 In one embodiment, hybrids of at least two peptides may be produced as recombinant fusion polypeptides which are expressed in any suitable expression system. The polypeptides may bind the receptor as either fusion constructs containing amino acid sequences besides the ligand binding sequences or as cleaved proteins from which signal sequences or other
30 sequences unrelated to ligand binding are removed. Sequences for facilitating purification of the fusion protein may also be expressed as part of

REPRESENTATIVE SITE 2 PEPTIDES (C-C LOOPS)

F8-derived (Long C-C loop):

	Clone	Sequence
5	F8	HLCVLEELFWGASLFGYCSG
	F8-C12	FQSLLEELVWGAPLFRYGTG
	F8-Des2	PLCVLEELFWGASLFGYCSG
10	F8-F12	PLCVLEELFWGASLFGQCSG
	F8-B9	HLCVLEELFWGASLFGQCSG
	F8-B12	DLRVLCLEFGGAYVLGYCSE
15	NNKH-2B3	HRSVLKQLSWGASLFGQWAG
	NNKH-2F9~	HLSVGEELSWWVALLGQWAR
	NNKH-4H4~	APVSTEELRWGALLFGQWAG

D8-derived (Small C-C loop):

	Clone	Sequence
20	D8	KWLDQEWAWVQCEVYGRGCP SKK
	D8-G1	QLEEEWAGVQCEVYGRECP S
	D8-B5~	ALEEEWAWVQVRSIRSGLP L
	D8-A7	SLDQEWAWVQCEVYGRGCL S
25	D8-F1~	WLEHEWAQIQCELYGRGCT Y

Midi C-C loop:

	Clone	Sequence
30	D8-F10	GLEQGC PWVGLEVQCRGCP S
	F8-B12~	DLRVLCLEFGGAYVLGYCSE
	F8-A9	PLWGLCELF GGASLFGYC S S

**Based on analysis of entire panning data, amino acid preferences at each position were calculated to define these "idealized" peptides.

* Peptides synthesized and currently being purified

~ Peptides planned

F. Multivalent Ligands

This invention provides ligands which preferentially bind different sites on IR and IGF-1R. The amino acid motifs which bind IR at one site (Site 1, Figure 13) are A6, B6, revB6, and F2. A second in site (Site 2, Figure 13) binds F8 and D8. Accordingly, multimeric ligands may be prepared according to the invention by covalently linking amino acid sequences. Depending on the purpose intended for the multivalent ligand, amino acid sequences which bind the same or different sites may be combined to form a single molecule. Where the multivalent ligand is constructed to bind to the same corresponding site on different receptors, or

REPRESENTATIVE SITE 1 PEPTIDES

A6-like (FYxWF):

	Clone	Sequence
5	G3	KRGGGTFYEWFEALRKHGAGKK
	H2	VTFTSAVFHENFYDWFVRQVSKK
	H2C	FHENFYDWFVRQVSKK
	A6S-IR3-E12	GRVDWLQRNANFYDWFVAELG
	A6S-IR4-G1	NGVERAGTGDNFYDWFVAQLH
10	H2CB-R3-B12	QSDSGTVHDRFYGWFRDTWAS
	20E2A-R3-B11	GRFYGWFDQDAIDQLMPWGFDP
	rB6-F6	RYGRWGLAQQFYDWFDR
	E4D α -1-B8-IR-	GFREGQRWYWFVAQVT
15	H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV
	H2CB-R3-D2	WTDVDGFHSGFYRWFQNWQER
	H2CB-R3-D12	VASGHVLHGQFYRWFVDQFAL
	H2CB-R4-H5	QARVGNVHQQFYEWFREVMQG
	H2C-B-E8*	TGHRRLGLDEQFYWWFRDALSG
20	H2CB-3-B6-IR-	VGDFCVSHDCFYGWFLRESMQ
	A6S-IR2-C1	RMFYSTGAPQNFYDWFVQEW

B6-like (FYxxLxxL):

	Clone	Sequence
25	20C11	KDRAFYNGLRDLVGAVYGAWDKK
	20E2	DYKDFYDAIDQLVRGSARAGGTRDKK
	B62-R3-C7	EHWNTVDPFFYFTLFEWLRESG
	B62-R3-C10	EHWNTVDPFFYQYFSELLRESG
30	20E2B-3-B3-IR	AGVNAGFYRYFSTLLDWDQOG
	20E2-B-E3*	IQGWEFFYGWFDVVAQMFE
	20E2A-R4-F9	PPWGARFYDAIEQLVFDNLCC
	RPNN-4-G6-HOLO*	RWPNFYGYFESLLTHFS
	RPNN-4-F3-HOLO*	HYNAFYEFQVLLAETW
35	20E2A-R4-E2	IGRVRSFYDAIDKLFQSDWER
	RPNN-2-C1-IR*	EGWDFYSYFSGLLASVT
	20E2B-4-F12-IR	SVKEVQFYRYFYDLLQSEESG
	20E2-B-E12	GNSGGSFYRYFQLLLDSDGMS
	20E2A-R3-B6	RDAGSSFYDAIDQLVCLTYFC
40		

Reverse B6-like (LxxLxxYF):

	Clone	Sequence
	rB6-A12	LDALDRLMRYFEERPSL
	rB6-F9	PLAELWAYFEHSEQGRSSAH
45		
	rB6-4-E7-IR	LDPLDALLQYFWSVPGH
	rB6-4-F9-IR	RGRLGSLSTQFYNWFAE
	rB6-E6	ADELEWLLDYFMHQPRP
	rB6-4-F12-IR	DGVLEELFSYFSATVGP
50		

Group 6 (WPxYxWL):

Clone	Sequence
R20 β -4-A4-IR	WPGYLFEEALQDWRGSTED

Peptides by design:**

Clone	Sequence
H2C-PD1-IR-	AAVHEQFYDWFADQYKK
A6S-PD1-IR-	QAPSNFYDWFVREWDKK
20E2-PD1-IR-	QSFYDYIEELLGGEWKK
60 B6C-PD1-IR-	DPFYQGLWEWLRESGKK

Besides relative binding at IR or IGF-1R, relative efficacy at the cognate receptor is another important consideration for choosing a potential therapeutic. Thus, a sequence which is efficacious at IR but has little or no significant activity at IGF-1R may also be considered as an important IR
5 therapeutic, irrespective of the relative binding affinities at IR and IGF-1R.

A6 selectivity for IR may be enhanced by including glutamic acid in a carboxyl terminal extension at position X₉₅. IR selectivity of the B6 motif may be enhanced by having a tryptophan or phenylalanine at X₁₁. Tryptophan at X₁₃ also favors selectivity of IR. A tryptophan amino acid at
10 X₁₃ rather than leucine at that position also may be used to enhance selectivity for IR. In the reverse B6 motif, a large amino acid at X₁₅ favors IR selectivity. Conversely, small amino acids may confer specificity for IGF-1R. In the F8 motif, an L in position X₂₃ is essentially required for IR binding. In addition, tryptophan at X₃₁ is also highly preferred. At X₃₂, glycine is
15 preferred for IR selectivity.

E. Multiple Binding Sites On IR And IGF-1R

The competition data disclosed herein reveals that at least two separate binding sites are present on IR and IGF-1R which recognize the different sequence motifs provided by this invention.

20 As shown in Figure 13, competition data (See Example 15) indicates that peptides comprising the A6, B6, revB6, and F2 motifs compete for binding to the same site on IR (Site 1) whereas the F8 and D8 motifs compete for a second site (Site 2). Similarly, the decrease of dissociation of B6 motif peptide (20E2) from IGF-1R by a D8 ligand indicates multiple
25 interacting binding sites.

The identification of peptides which bind to separate binding sites on IR and IGF-1R provides for various schemes of binding to IR or IGF-1R to increase or decrease its activity. Examples of such schemes for IR are illustrated in Figure 15.

30 The table below shows sequences based on their groups, which bind to Site 1 or Site 2.

MOTIF 10 (Group6):

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
R20β-4-E8-IR	VRGFQGGTVWPGYEWLRNAA	41.0	34.9	9.7	0.1
40F-4-D1-IGFR	LSCLAYSRHGIWRPSTDGLGRSVGEGSVSTRWRGYDWF	4.9	4.6	13.1	0.1
40F-4-B1-IGFR	GLDHSDAVGVHLGFAPPAQARGRWEAGGLEDTWAGYDNL	4.1	3.0	13.1	0.1
40F-4-D10-IGFR	W.GYAWLS	4.9	4.5	11.7	0.1
R20β-4-E8-IR	VRGFQGGTVWPGYEWLRNAA	41.0	3.6	0.1	9.7

Clon	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
B6H α -3-E8-IR	RGKTAAVIVGRPADPFYHKLSELLQGG	47.6	5.3	4.8	0.2
B6H α -3-F10-IR	GCVVEWQKWHGASDPFYHKLSELGGCS	47.2	8.8	4.6	0.2
B6H α -2-D6-IR	GRTMAVMAAGPDDPFYHKLSELVQGG	33.5	4.4	4.4	0.2
B6H α -3-E7-IR	GCAVVEEAERSRGDPFYHKLSELIQGC	47.0	5.6	4.3	0.2
B6H α -2-D1-IR	GCEVIVEEGDSADPFYHKLSELCOGS	11.7	5.4	4.2	0.2
20E2A-3-D10-IGFR	MMVVDGFDALHQLVVAQSLG	20.6	6.9	3.9	0.3
20E2A-3-A12-IGFR	LSVALSFYDALGQLVAGEGRW	16.1	4.3	3.9	0.3
B6H α -4-G8-IR	GGTKAVAKVGRDDPFYHKLSELLQGS	32.3	6.1	3.6	0.3
B6L-4-D7-IR	AETSVQVGWIRLQSVWPGHEWNTVDPFYHKLSELLRGSGA	14.3	4.8	3.4	0.3
B6H α -1-A3-IR	SRAKVEAEMPDSGDPFYHKLSELLASG	37.4	2.6	3.3	0.3
B6H α -3-F7-IR	SRVAATKEKRPSDDPFYHKLSELLQGS	41.5	3.1	3.1	0.3
B6H α -2-D8-IR	SSETAKMVTGTRDDPFYHKLSELVQGS	19.3	3.0	3.0	0.3
B6H α -1-B3-IR	GCITAENGAGDPFYHKLSELGGCS	33.1	3.2	2.9	0.3
B6H α -3-E5-IR	RCGDEEGWQENRRDDPFYHKLSELFQGC	28.8	2.9	2.9	0.3
20E2A-4-G11-IGFR	MNVFVSFYDAIDQLVCQRIGC	20.7	3.3	2.6	0.4
20E2B β -3-C7-IR	QSGSGDFYDWLSRLIRGNMGD	1.5	3.1	2.0	0.5
B6H α -3-E6-IR	CGAKMTGTPNDPFYHKLSELLQRG	18.2	2.3	1.9	0.5
20E2A-3-A3-IGFR	GHYFGSFYDAIDQLVAGMLPG	5.2	3.0	1.9	0.5
B6L-4-A7-IR	AGTPAQVG*NRLWSVWPGHEWNTVDPFYHKLSELLRESGA	11.6	3.4	1.8	0.6
B6H α -3-F1-IR	CSMAAFAEAGDDDDPFYHKLSELCOGS	22.5	2.4	1.8	0.5
B6L-3-G6-IR	VDTPAQVGNRLWSVWPGHEHWYTTDDPFYH*LSELLRESGA	7.6	2.5	1.4	0.7
B6L-3-G5-IR	AETSAQVGWQRLWSVWPGDHWSTLDPFYHKLSELLRESGA	11.5	2.0	1.4	0.7
20E2A-3-A4-IGFR	AGSVTSFYDAMEQLVATGTSA	16.8	2.5	1.4	0.7
B6-PD1-IGFR	TDDGFDALQLVQGSKK				
20E2-PD1-IGFR (RP10)	GSFYDALQLVGEQGGK				

-40-

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
20E2B-1-A11-IGFR	RFSTDGFYQYLLALVGGPVG	15.0	9.5	9.7	0.1
20E2B-3-D4-IGFR	NSRDGGFYQLERLLGFPVTG	8.1	7.9	9.6	0.1
20E2B-2-B11-IGFR	VVTPVNFYRALEALVRG.RLG	13.9	10.6	9.4	0.1
20E2B-3-C8-IGFR	QPAPDGFYSALMKLIGRGVS	18.5	15.6	8.9	0.1
20E2B-2-B2-IGFR	PGTDLGFYQALRCVVIQACD	11.7	4.9	8.1	0.1
20E2B-4-F10-IGFR	AQPCGGFYGLLEQLVGRSVCD	19.0	17.3	7.8	0.1
20E2B-4-F9-IGFR	QPDHSYFYSLLQELVGSEERL	11.9	14.7	7.7	0.1
20C-3-A4-IGFR	QFYGCLLDLSLGVPSFGWRRRCITA	17.7	8.8	7.6	0.1
20E2B-3-D11-IGFR	LGVTDFGYAALGYLIHGVGQF	14.3	12.2	7.6	0.1
20E2B-3-C11-IGFR	CMM.DGFYAGLGCLLTAGEGR	15.3	15.4	7.5	0.1
20E2B-2-B3-IGFR	ICTGQGFYQVLCGLLRGTSAR	9.1	5.3	7.4	0.1
20E2B-3-D12-IGFR	QGNVDFYGWIGRLLAKQGS	10.3	6.2	7.3	0.1
20E2B-3-E12-IGFR	VATSQGFYSGLSELLQGGNV	13.9	6.0	7.3	0.1
20E2B-2-B8-IGFR	IWATGDFYRLLSQLVMGRVGT	17.4	5.7	7.2	0.1
NNRPγ-4-A9-IR	EGSGFYGYFFSLLGLQG	3.0	10.0	7.1	0.1
20E2B-4-G11-IGFR	RQGTGSFYLMLEQLLVGARGP	8.9	4.5	7.0	0.1
20E2B-3-D6-IGFR	DSVGDNFYQLLESVLVGGHVG	20.7	17.8	6.9	0.1
B6Hα-2-C7-IR	RGIVAMVEATEVGSDDHPFYHKLSELLVQGS	45.1	6.7	6.7	0.1
20E2B-2-B7-IGFR	LSSDGGFYRALNLLQGSAGR	18.0	6.1	6.7	0.1
20E2B-3-C4-IGFR	ASSASGFYELLQRLAGLGLEV	23.4	20.4	6.2	0.2
20C-3-E4-IGFR	FFYRCLSRLLGGQLGSRLLGLSCIGD	37.7	7.7	6.0	0.2
NNRPγ-4-A1-IR	IIGGFYSYFNSVLRIGT	9.7	10.9	6.0	0.2
20E2B-4-H8-IGFR	PAGPCGFYCGLLGLHGDQSP	7.2	5.3	5.9	0.2
20E2B-4-H9-IGFR	RCQGTGFYTCIQELIGFGDPD	4.5	5.2	5.6	0.2
B6Hα-2-C10-IR	SGAKVIVVTGSDGDPFYHKLSELLQGS	46.9	5.8	5.3	0.2
20E2A-3-C7-IGFR	VGTVAGFYDAIAQLVARASRV	17.6	5.4	5.1	0.2
20E2B-1-A8-IGFR	TLRSPTFYDWLEMLVTHGQGG	16.1	4.4	5.0	0.2
NNRPγ-4-A7-IR	RFDPFYSYFVNLLGASA	2.5	6.3	4.9	0.2

MOTIF 2 (B6-like):

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
20C-3-G3-IGFR	TFYSCIASLITGTTPQPNRGPWRCR	33.1	32.3	27.0	<0.1
20C-4-C7-IGFR	FFYDCIAALLQGVARYHDLCAVEIT	35.3	28.0	21.8	<0.1
B6H α -1-B5-IR	CCTTEMVMDARDDPFYHKLSELVTGG	41.5	20.5	20.5	0.0
R20 β -4-A6-IR	RGQSDAFYSGLWALLGLSDG	9.3	25.9	17.3	0.1
20E2B-1-A6-IGFR	GVRAMSFYDALVSVLGLPSG	18.6	18.1	16.8	0.1
R20 α -4-20A12-IR	RLFYCGIQALGANLGYSGCV	48.6	39.9	16.6	0.1
20E2B β -4-G7-IR	LQPCSGFYECIERLLIGVKLSG	19.9	25.2	15.8	0.1
NNRPY-4-B11-IR	LKDGFDYDFWQRLHLGS	4.1	18.7	15.5	0.1
20E2B-3-C6-IGFR	VEGRGLFYDLLRQLLARRQNG	17.9	16.8	14.8	0.1
B6H α -1-A2-IR	RCNDDGGKGWSDDPFYHKLSELICGG	22.3	14.6	14.6	0.1
20E2A-4-F11-IGFR	QGGASAFYDAIDRLLRMRIGG	21.3	18.8	14.6	0.1
B6H α -3-E9-IR	RCEEKQAEVGPSSDPFYHKMSEILGCR	44.6	24.2	14.2	0.1
20C-3-F6-IGFR	DRDFCRFYERLTALVGGQVDGGWPC	33.5	26.1	14.1	0.1
20E2B-4-H3-IGFR	KLHNLMPYQGLQRLVWGAGLG	11.2	14.8	13.9	0.1
20E2B-3-C2-IGFR	GNGDGMFYQLLSLLVGRDMHV	13.1	8.9	13.8	0.1
20C-3-A1-IGFR	SSYGCDGFYLMFLSLGLVASQELEC	26.5	20.8	13.7	0.1
20E2B-3-E3-IGFR	PDLHKGFYAQLAQLIRGQLLS	22.4	16.3	13.1	0.1
R20 α -3-20E2-IR	FYDAIDQLVRGSARAGGTRD	46.3	39.9	12.9	0.1
20E2B-4-H12-IGFR	YSCGDGFYSLSDLLGGQFRC	6.5	9.7	12.8	0.1
B6H α -3-F11-IR	RGMKEEVLVGGSTDPFYHKLSELQGS	49.5	18.7	11.7	0.1
20E2B-3-D2-IGFR	IQQELTFYDLLHRLVRSELGS	20.7	12.4	11.7	0.1
20E2B-3-D8-IGFR	GGTEVDFYRALERLVRGQLGL	20.4	17.7	11.3	0.1
20E2B-3-E8-IGFR	LRIANLFYQRLWDIAFGGGG	15.7	16.7	11.1	0.1
B6H α -2-C4-IR	RCGRW*AEIMGAGDDPFYHKLSELVCG	20.7	9.9	11.0	0.1
R20 α -4-20C11-IR	DRAFYNGLRDLVGAVYGAWD	43.7	30.8	10.3	0.1
20E2B-4-F8-IGFR	PVGVGQGFYEGLSRLVLGRGGW	12.3	7.3	9.7	0.1

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IR	IGF-1R/IR
H2CA-2-B2-IGFR	GAPVDQLHEDFYDWFVRQVSQAATG	4.1	3.4	1.0	3.5
E4Dα-2-D11-IR	GFREGSFYDWFQAQVT	40.2	11.1	3.3	3.4
20E2Bβ-4-G6-IR	SQAGSAFYAWFDQVLRTHSA	22.4	6.2	1.9	3.3
H2CA-4-H9-IGFR	RGAVAGFHDQFYDWFDRQVSRVHKFG	8.7	5.6	1.9	3.0
H2CA-2-B11-IGFR	ATCDAGFHEHFYDWFALQVSDCGRQS	11.9	4.6	1.6	3.0
H2CA-3-E8-IGFR	LGYQEPFQQNFYDWFVRQVSGAENAG	13.2	6.3	2.2	2.9
A6S-2-D11-IR	EAAASLGSDRNFFYDWFVRQVV	48.4	37.4	13.5	2.8
A6S-2-D1-IR	VERSASSQDGNFYDWFVQIR	37.8	30.6	12.0	2.6
A6S-3-E2-IR	TSEVQRRSQDNFYDWFVAQVA	33.1	24.7	9.8	2.5
H2CA-3-E11-IGFR	HLADGQFHEKFYDWFERQISSRCNDC	4.7	2.2	1.0	2.2
H2CA-3-C11-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3.3	1.6	2.1
A6-PD1-IGFR	SFHEDFYDWFDRQVSGSLKK				
H2C-PD1-IGFR (RP9)	GSILDESFYDWFERQLGKK				

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IGF-1R-SELECTIVE SEQUENCES

MOTIF 1 (A6-like):

Clone	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF-1R	IGF-1R/IR	IR/IGF-1R
A6L-0-E6-IR	YRGLVLGRSSDGAGKVAERPARIGQTVFAVNFYDWFV	31.0	31.0	17.0	0.1
H2CA-4-G9-IGFR	GIISQSCPESFYDWFAGQVSDPWWCW	8.6	9.5	16.0	0.1
H2CA-4-H6-IGFR	VGRASGFPENFYDWFGRQLSLQSGEQ	4.9	10.5	14.6	0.1
A6L-0-E4-IR	YRGLVLGRISDCAG#VASEPPARIGRKVFAVNFYDWFV	26.0	16.0	13.0	0.1
A6L-0-H3-IR	YRGLVLGRISGAGKAASERPARIGQKVSANFYDWFV	27.0	26.0	13.0	0.1
H2CA-4-F5-IGFR	VGYQGQGDENFYDWFIRQVSGRLGVQ	5.5	9.7	12.3	0.1
H2CA-4-H8-IGFR	SACQFDCHENFYDWFARQVSGGAAYG	5.6	9.2	9.4	0.1
H2CA-4-F11-IGFR	SAAQLFFQESFYDWFELRQVAESSQPN	3.5	6.8	6.7	0.1
H2CA-4-F6-IGFR	AVRATRFDEAFYDWFVRQISDGQGNK	3.9	7.3	6.4	0.2
H2CA-4-F10-IGFR	VNQSGSIHENFYDWFVRQVSHQGVNR	4.9	5.7	5.9	0.2
H2CA-1-A3-IGFR	APDPSDFQELFYDWFVRQVSRMPGGG	7.7	3.8	5.1	0.2
H2CA-3-C8-IGFR	SSCDGAGHESFYEMFVRQVSGCRSV	15.1	5.6	4.8	0.2
H2CA-2-B9-IGFR	RAGSSDFHEDFYEMFVRQVSLSLKKG	9.3	7.0	4.2	0.2
H2CA-4-H4-IGFR	QAVQPGFHEEFYDWFVRQVSTGVGGG	3.9	4.1	4.2	0.2
E4DQ-4-H2-IR	GFREGNFYEWFAQQVT	37.8	33.9	4.1	0.2
H2CA-4-F7-IGFR	SSIGGFHENFYDWFSRQLSQSPPLK	1.5	3.2	4.1	0.2
H2CA-3-D6-IGFR	QSPVGSSEDFYDWFVRQVAQSGAHQ	8.3	9.0	4.0	0.3
H2CA-3-D8-IGFR	NYRRQVFNENFYDWFDRQVFSLVTPG	10.9	7.2	4.0	0.3
H2CA-4-G11-IGFR	TLDGGSFEEQFYDWFVRQLSYRTNPD	10.8	9.5	3.9	0.3
H2CA-4-F1-IGFR	FYVQWGHENFYDWFDRQVSSGGAG	5.8	3.5	3.8	0.3
H2CA-3-D7-IGFR	LRRQAPVEENFYDWFVRQVSGDRVGG	13.3	3.0	3.7	0.3
H2CA-1-A7-IGFR	RCGRELYHSTFYDWFDRQVAGRTCPS	8.0	2.2	3.7	0.3
H2CA-2-B4-IGFR	CCLLCRFQQNFYDWFVCCGISRLRPL	3.5	4.1	3.6	0.3
H2CA-2-B3-IGFR	PPLASDLDVQFYGFVFWQVSPPPGRGG	7.7	3.8	3.6	0.3

helix, it is important to maintain the spacing of those residues along the amino acid sequence. For example, the second and third amino acids of B6 (X₇ and X₈) are oriented at opposite sides of the helix. See Figure 12.

D. IR Binding Preferences

5 As indicated above, the amino acid sequences containing the motifs of this invention may be constructed to have enhanced selectivity for either IR or IGF-1R by choosing appropriate amino acids at specific positions of the motifs or the regions flanking them. By providing amino acid preferences for IR or IGF-1R, this invention provides the means for constructing amino acid
10 sequences with minimized activity at the non-cognate receptor. For example, the amino acid sequences disclosed herein with high affinity and activity for IR and low affinity and activity for IGF-1R are desirable as IR agonist as their propensity to promote undesirable cell proliferation, an activity of IGF-1 agonists, is reduced. Ratios of IR binding affinity to IGF-1R
15 binding affinity for specific sequences are provided in Figures 1A-10I. As an insulin therapeutic, the IR/IGF-1R binding affinity ratio is preferably greater than 100. Conversely, for use as an IGF-1R therapeutic, the IR/IGF-1R ratio should be less than 0.01. Examples of peptides that selectively bind to IGF-1R are shown below.

20

2. Formula 2

B6 with amino terminal and carboxy terminal extensions may be represented as $X_{98} X_{99} B6 X_{100}$. X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine, and proline. The presence of an aspartic acid at X_{98} and a proline at X_{99} is associated with an enhancement of binding for both IR and IGF-1R. A hydrophobic amino acid is preferred for the amino acid at X_{100} , an aliphatic amino acid is more preferred. Most preferably leucine, for IR and valine for IGF-1R. Negatively charged amino acids are preferred at both the amino and carboxy terminals of Formula 2A.

3. Formula 3

An amino terminal extension of Formula 3 defined as $X_{101} X_{102} X_{103}$ revB6 wherein X_{103} is a hydrophobic amino acid, preferably leucine, isoleucine or valine, and X_{102} and X_{101} are preferably polar amino acids, more preferably aspartic acid or glutamic acid may be useful for enhancing binding to IR and IGF-1R. No preference is apparent for the amino acids at the carboxy terminal end of Formula 3.

C. Secondary Structure

Without being bound by theory, it is believed that the B6 and reverse B6 motifs participate in alpha helix formation such that the most highly preferred residues at positions X_6, X_7, X_{10} and X_{13} (B6) and $X_{14} X_{17} X_{20}$ and X_{21} (rB6) reside on the same side of a helix. See Figure 12. Because both B6 and RB6 motifs form structurally analogous motifs from their palindrome sequences, the use of D-amino acids instead of typical L-amino acids would be expected to produce amino acid sequences having similar properties to the L-amino acid sequences. D-amino acids may be advantageous, as the resultant sequences may be more resistant to enzymatic degradation than L-amino acid sequences. In addition, to maintain the appropriate orientation of highly preferred amino acid sequences on the appropriate side of the

preferred IR or IGF-1R consensus sequences which do not correspond to one of the motifs described above. Such sequences are described in Figures 10C-10I.

5 **B. Amino And Carboxyl Terminal Extensions
 Modulate Activity of Motifs**

In addition to the motifs stated above, the invention also provides preferred sequences at the amino terminal or carboxyl terminal ends which are capable of enhancing binding of the motifs to either IR, IGF-1R, or both. In addition, the use of the extensions described below does not preclude the
10 possible use of the motifs with other substitutions, additions or deletions which allow for binding to IR, IGF-1R or both.

1. Formula 1

Any amino acid sequence may be used for extensions of the amino terminal end of A6, although certain amino acids in amino terminal
15 extensions may be identified which modulate activity. Preferred carboxy terminal extensions for A6 are A6 X₉₃ X₉₄ X₉₅ X₉₆ X₉₇ wherein X₉₃ may be any amino acid, but is preferably selected from the group consisting of alanine, valine, aspartic acid, glutamic acid, and arginine, and X₉₄ and X₉₇ are any amino acid; X₉₅ is preferably glutamine, glutamic acid, alanine or
20 lysine but most preferably glutamine. The presence of glutamic acid at X₉₅ however may confer some IR selectivity. Further, the failure to obtain sequences having an asparagine or aspartic acid at position X₉₅ may indicate that these amino acids should be avoided to maintain or enhance sufficient binding to IR and IGF-1R. X₉₆ is preferably a hydrophobic or
25 aliphatic amino acid, more preferably leucine, isoleucine, valine, or tryptophan but most preferably leucine. Hydrophobic residues, especially tryptophan at X₉₆ may be used to enhance IR selectivity.

X₁₁₄) ends immediately flanking X₁₀₇-X₁₁₁ are preferably a cysteine residue, most preferably at X₁₀₅ and X₁₁₃ respectively. Without being bound by theory, the cysteines are preferably spaced so as to allow for the formation of a loop structure. X₁₀₄ and X₁₁₄ are both small amino acids such as, for example, alanine and glycine. Most preferably, X₁₀₄ is alanine and X₁₁₄ is glycine. X₁₀₅ may be any amino acid but is preferably valine. X₁₁₂ is preferably asparagine. Thus, the most preferred sequence is ACVWPTYWNCG. The IR binding displayed amino acid sequences are described in Figure 8.

10 9. An amino acid sequence comprising
DYKDLQCQSWGVRIGWLAGLCPKK (Formula 9, JBA5). The Formula 9 motif is another motif believed to form a cysteine loop which possesses agonist activity at both IR and IGF-1R. Although IR binding is not detectable by ELISA, binding of Formula 9 to IR is competed by insulin and is agonistic.
15 See Figure 11A. Binding of Formula 9 through IGF-1R is detected by ELISA. Nonlimiting examples of Formula 9 amino acid sequences are described in Figures 9A-9C.

 10. WX₁₂₃ GYX₁₂₄ WX₁₂₅ X₁₂₆ (Formula 10, Group 6 Secondary Library) wherein X₁₂₃ is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X₁₂₄ is any amino acid, but preferably a charged or aromatic amino acid; X₁₂₅ is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X₁₂₆ is any amino acid, but preferably a small amino acid. Nonlimiting examples of Formula 10 amino acid sequences are described in Figures 10A-10B.

25 11. Other Motifs

Another motif for use with this invention includes WPGY. Examples of specific peptide sequences comprising this motif include KVRGFQGGTVWPGYEWLRNAKK (E8), and KSMFVAGSDRWPGYGVLDWLKK (F2).

30 Various amino acid sequences which bind IR and/or IGF-1R have been identified through panning of various libraries designed to identify

most useful as an amino acid sequence having a preference for binding to IR as only a few D8 sequences capable of binding to IGF-1R over background have been detected. A preferred sequence for binding to IR is X₆₂ L X₆₄ X₆₅ X₆₆ W X₆₈ X₆₉ X₇₀ X₇₁ C X₇₃ X₇₄ X₇₅ X₇₆ X₇₇ X₇₈ C X₈₀ X₈₁.

- 5 Nonlimiting examples of Formula 6 amino acid sequences are described in Figures 6A-6E.

7. HX₈₂, X₈₃, X₈₄ X₈₅ X₈₆ X₈₇ X₈₈ X₈₉ X₉₀ X₉₁ X₉₂ (Formula 7)

wherein X₈₂ is proline or alanine but most preferably proline; X₈₃ is a small amino acid more preferably proline, serine or threonine and most preferably
10 proline; X₈₄ is selected from leucine, serine or threonine but most preferably leucine; X₈₅ is a polar amino acid preferably glutamic acid, serine, lysine or asparagine but more preferably serine; X₈₆ may be any amino acid but is preferably a polar amino acid such as histidine, glutamic acid, aspartic acid, or glutamine; X₈₇ is an aliphatic amino acid preferably leucine, methionine or
15 isoleucine and most preferably leucine; amino acid X₈₈, X₈₉ and X₉₀ may be any amino acids; X₉₁ is an aliphatic amino acid with a strong preference for leucine as is X₉₂. Phenylalanine may also be used at position 92. A preferred consensus sequence of Formula 7 is HPPLSX₈₆LX₈₈X₈₉X₉₀LL. The Formula 7 motif binds to IR with little or no binding to IGF-1R.

- 20 Nonlimiting examples of Formula 7 amino acid sequences are described in Figure 7.

8. Another sequence is X₁₀₄, X₁₀₅ X₁₀₆ X₁₀₇ X₁₀₈ X₁₀₉ X₁₁₀ X₁₁₁

X₁₁₂ X₁₁₃ X₁₁₄. (Formula 8) which comprises eleven amino acids wherein at least one, and preferably two of the amino acids of X₁₀₆ through X₁₁₁ are
25 tryptophan. In addition, it is also preferred that when two tryptophan amino acids are present in the sequence they are separated by three amino acids, which are preferably, in sequential order proline, threonine and tyrosine with proline being adjacent to the tryptophan at the amino terminal end.

Accordingly, the most preferred sequence for X₁₀₇ X₁₀₈ X₁₀₉ X₁₁₀ X₁₁₁ is
30 WPTYW. At least one of the three amino acids on the amino terminal (X₁₀₄, X₁₀₅ X₁₀₆) and at least one of the amino acids carboxy terminal (X₁₁₂ X₁₁₃

acids are defined above. A more preferred F8 sequence is HLCVLEELFWGASLFGYCSG ("F8"). Amino acid sequences comprising the F8 sequence motif preferably bind to IR over IGF-1R. Figures 4A-4E list nonlimiting examples of Formula 4 amino acid sequences.

- 5 5. $X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} X_{56} X_{57}$
 $X_{58} X_{59} X_{60} X_{61}$ ("mini F8", Formula 5) wherein X_{42} , X_{43} , X_{44} , X_{45} , X_{53} , X_{55} , X_{56} ,
 X_{58} , X_{60} and X_{61} are any amino acid. X_{43} , X_{46} , X_{49} , X_{50} and X_{54} are
hydrophobic amino acids, however, X_{43} and X_{46} are preferably leucine,
whereas X_{50} is preferably phenylalanine or tyrosine but most preferably
10 phenylalanine. X_{47} and X_{59} are cysteines. X_{48} is preferably a polar amino
acid, i.e. aspartic acid or glutamic acid, but most preferably glutamic acid.
Use of the small amino acid at position 54 may confer IGF-1R specificity.
 X_{51} , X_{52} and X_{57} are small amino acids, preferably glycine. A preferred
consensus sequence for mini F8 is $X_{42} X_{43} X_{44} X_{45} \text{LCEX}_{49} \text{FGGX}_{53} X_{54} X_{55}$
15 $X_{56} \text{GX}_{58} \text{CX}_{60} X_{61}$. Amino acid sequences comprising the sequence of
Formula 5 preferably bind to IGF-1R or IR. Nonlimiting examples of
Formula 5 amino acid sequences are described in Figure 5.

6. $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77}$
 $X_{78} X_{79} X_{80} X_{81}$ (Formula 6, "D8") wherein X_{62} , X_{65} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} ,
20 X_{77} , X_{78} , X_{80} and X_{81} may be any amino acid. X_{66} may also be any amino
acid, however, there is a strong preference for glutamic acid. Substitution of
 X_{66} with glutamine or valine may result in attenuation of binding. X_{63} , X_{70} ,
and X_{74} are hydrophobic amino acids. X_{63} is preferably leucine, isoleucine,
methionine, or valine, but most preferably leucine. X_{70} and X_{74} are
25 preferably valine, isoleucine, leucine, or methionine. X_{74} is most preferably
valine. X_{64} is a polar amino acid, more preferably aspartic acid or glutamic
acid, and most preferably glutamic acid. X_{67} and X_{75} are aromatic amino
acids. Whereas tryptophan is highly preferred at X_{67} , X_{75} is preferably
tyrosine or tryptophan but most preferably tyrosine. X_{72} and X_{79} are
30 cysteines which again are believed to form a loop which position amino acid
may be altered by shifting the cysteines in the amino acid sequence. D8 is

acid is strongly preferred at X₁₈. See Figures 3A-3D for nonlimiting examples of Formula 3 amino acid sequences.

4. X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀X₄₁
 (Formula 4, "F8") wherein X₂₂, X₂₅, X₂₆, X₂₈, X₂₉, X₃₀, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇,
 5 X₃₈, X₄₀, and X₄₁ are any amino acid. X₃₅ and X₃₇ may be any amino acid
 when the F8 motif is used as an IR binding ligand or as a component of an
 IR binding ligand, however for use as an IGF-1R binding ligand, glycine is
 strongly preferred at X₃₇ and a hydrophobic amino acid, particularly, leucine,
 is preferred at X₃₅. X₂₃ is a hydrophobic amino acid. Methionine, valine,
 10 leucine or isoleucine are preferred amino acids for X₂₃, however, leucine
 which is most preferred for preparation of an IGF-1R binding ligand is
 especially preferred for preparation of an IR binding ligand. At least one
 cysteine is located at X₂₄ through X₂₇, and one at X₃₉ or X₄₀. Together the
 cysteines are capable of forming a cysteine cross-link to create a looped
 15 amino acid sequence. In addition, although a spacing of 14 amino acids in
 between the two cysteine residues is preferred, other spacings may also be
 used provided binding to IGF-1R or IR is maintained. Accordingly, other
 amino acids may be substituted for the cysteines at positions X₂₄ and X₃₉ if
 the cysteines occupy other positions. In one embodiment, for example, the
 20 cysteine at position X₂₄ may occur at position X₂₇ which will produce a
 smaller loop provided that the cysteine is maintained at position X₃₉. These
 smaller looped peptides are described herein as Formula 5, infra. X₂₇ is any
 polar amino acid, but is preferably selected from glutamic acid, glutamine,
 aspartic acid, asparagine, or as discussed above cysteine. The presence of
 25 glutamic acid at position X₂₇ decreases binding to IR but has less of an
 effect on binding to IGF-1R. X₃₁ is any aromatic amino acid and X₃₂ is any
 small amino acid. For binding to IGF-1R, glycine or serine are preferred at
 position X₃₁, however, tryptophan is highly preferred for binding to IR. At
 position X₃₂, glycine is preferred for both IGF-1R and IR binding. X₃₆ is an
 30 aromatic amino acid. A preferred consensus sequence for F8 is X₂₂ LC X₂₅
 X₂₆ E X₂₈ X₂₉ X₃₀ WG X₃₃ X₃₄ X₃₅ X₃₆ X₃₇ X₃₈ C X₄₀ X₄₁ whereas the amino

preferably glycine, aspartic acid, glutamic acid, asparagine or alanine. More preferably X_{117} is glycine, aspartic acid, glutamic acid and asparagine whereas X_{118} is more preferably glycine, aspartic acid, glutamic acid or alanine.

5 X_8 when present in the Formula 2A motif is preferably arginine, glycine, glutamic acid, or serine.

X_{11} when present in the Formula 2A motif is preferably glutamic acid, asparagine, glutamine, or tryptophan, but most preferably glutamic acid.

10 X_{12} when present in the Formula 2A motif is preferably aspartic acid, glutamic acid, glycine, lysine or glutamine, but most preferably aspartic acid.

X_{119} is preferably glutamic acid, glycine, glutamine, aspartic acid or alanine, but most preferably glutamic acid.

X_{120} is preferably glutamic acid, aspartic acid, glycine or glutamine, but most preferably glutamic acid.

15 X_{121} is preferably tryptophan, tyrosine, glutamic acid, phenylalanine, histidine, or aspartic acid, but most preferably tryptophan or tyrosine.

X_{122} is preferably glutamic acid, aspartic acid or glycine; but most preferably glutamic acid.

20 Preferred amino acid residue are identified based on their frequency in clones over two fold over that expected for a random event, whereas the more preferred sequences occur about 3-5 times as frequently as expected.

Nonlimiting examples of amino acid sequences having the Formula 2 and 2A motifs are described in Figures 2A-2P.

3. $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ (Formula 3, reverse B6, revB6),
 25 wherein X_{14} and X_{17} are hydrophobic amino acids; X_{14} , X_{17} are preferably leucine, isoleucine, and valine, but most preferably leucine; X_{15} , X_{16} , X_{18} and X_{19} may be any amino acid; X_{20} is an aromatic amino acid, preferably tyrosine or histidine, but most preferably tyrosine; and X_{21} is an aromatic amino acid, but preferably phenylalanine or tyrosine, and most preferably
 30 phenylalanine. For use as an IGF-1R binding ligand, an aromatic amino

at IGF-1R, but agonist or antagonist activity at IR depending on the identity of amino acids flanking A6. See Figure 11A. Two amino acid sequences comprising the A6 motif possess agonist activity at IR are

FHENFYDWFVRQVSKK (D117; H2C) and

- 5 GRVDWLQRNANFYDWFVAELG-NH₂ (S175). Nonlimiting examples of Formula 1 amino acid sequences are shown in Figures 1A-1O.

2. X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃ (Formula 2, the B6 motif) wherein X₆ and X₇ are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X₆ is phenylalanine and X₇ is tyrosine. X₈, X₉, X₁₁ and X₁₂ may be any amino acid. X₁₀ and X₁₃ are hydrophobic amino acids, preferably leucine, isoleucine, phenylalanine, tryptophan or methionine, but more preferably leucine or isoleucine. X₁₀ is most preferably isoleucine for binding to IR and leucine for binding to IGF-1R. X₁₃ is most preferably leucine. Amino acid sequences of Formula 2 may function as an antagonist at the IGF-1R, or as an agonist at the IR. Preferred consensus sequences of the Formula 2 motif are FYX₈ X₉ L X₁₁ X₁₂L, FYX₈ X₉ IX₁₁ X₁₂ L FYX₈ AIX₁₁ X₁₂L, and FYX₈ YFX₁₁ X₁₂ L.

Another Formula 2 motif for use with this invention comprises FYX₈ YFX₁₁ X₁₂ L and is shown as Formula 2A ("NNRP") below:

- 20 X₁₁₅ X₁₁₆ X₁₁₇ X₁₁₈ FY X₈ YF X₁₁ X₁₂ L X₁₁₉ X₁₂₀ X₁₂₁ X₁₂₂, wherein X₁₁₅-X₁₁₈ and X₁₁₉-X₁₂₂ may be any amino acid which allows for binding to IR or IGF-1R. X₁₁₅ is preferably selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid and arginine. Aspartic acid, glutamic acid, glycine, and arginine are more preferred. Tryptophan is most preferred. The preference for tryptophan is based on its presence in clones at a frequency three to five fold higher than that expected over chance for a random substitution, whereas aspartic acid, glutamic acid and arginine are present about two fold over the frequency expected for random substitution.
- 30 X₁₁₆ preferably is an amino acid selected from the group consisting of aspartic acid, histidine, glycine, and asparagine. X₁₁₇ and X₁₁₈ are

The amino acids within each of these defined groups may be substituted for each other in the motifs described below, subject to the specific preferences stated herein. In addition, synthetic or non-naturally occurring amino acids may also be used in accordance with this invention.

5 Also included within the scope of this invention are amino acid sequences containing substitutions, additions, or deletions based on the teachings disclosed herein and which bind to IR or IGF-1R with the same or altered affinity. For example, amino acid residues located at the carboxy and amino terminal regions of the consensus motifs described below, which
10 amino acid residues are not associated with a strong preference for a particular amino acid, may optionally be deleted providing for truncated sequences. Certain amino acids such as lysine which promote the stability of the amino acids sequences may be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger
15 sequence which is soluble, or linked to a solid support.

Peptides that bind to IGF-1R, and methods and kits for identifying such peptides, have been disclosed by Beasley et al., U.S. Application Serial No. 09/146,127, filed September 2, 1998, which is incorporated by reference in its entirety.

20 A. Consensus Motifs

The following motifs have been identified as conferring binding activity to IR and/or IGF-1R:

1. $X_1X_2X_3X_4X_5$ (Formula 1, the A6 motif) wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most
25 preferably, X_1 and X_5 are phenylalanine and X_2 is tyrosine. X_3 may be any small polar amino acid, but is preferably selected from aspartic acid, glutamic acid, glycine, or serine, and is most preferably aspartic acid or glutamic acid. X_4 is most preferably tryptophan, tyrosine, or phenylalanine and most preferably tryptophan. Particularly preferred embodiments of the
30 A6 motif are FYDWF and FYEWF. The A6 motif possesses agonist activity

acid sequence preferably has affinity for the receptor of between about 10^{-5} to about 10^{-12} M.

A further consideration in identifying peptides provided by this invention for use as therapeutics is the relative activity at either IR or IGF-IR.

5 Thus, a peptide which has efficacy at IR and clinically insignificant activity of IGF-IR may be a useful therapeutic even though such a peptide may bind IGF-IR with relatively high affinity.

At least ten different binding motifs have been identified which bind to active sites on IR; at least four of these also bind to IGF-1R. The binding
10 motifs are defined based on the analysis of several different amino acid sequences and analyzing the frequency that particular amino acids or types of amino acids occur at a particular position of the amino acid sequence.

For the purposes of this invention, the amino acids are grouped as follows: amino acids possessing alcohol groups are serine (S) and
15 threonine (T). Aliphatic amino acids are isoleucine (I), leucine (L), valine (V), and methionine (M). Aromatic amino acids are phenylalanine (F), histidine (H), tryptophan (W), and tyrosine (Y). Hydrophobic amino acids are alanine (A), cysteine (C), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (L), methionine (M), arginine (R), threonine (T), valine
20 (V), tryptophan (W), and tyrosine (Y). Negative amino acids are aspartic acid (D) and glutamic acid (E). The following amino acids are polar amino acids: cysteine (C), aspartic acid (D), glutamic acid (E), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), and threonine (T). Positive amino acids are histidine (H), lysine (K), and arginine (R).
25 Small amino acids are alanine (A), cysteine (C), aspartic acid (D), glycine (G), asparagine (N), proline (P), serine (S), threonine (T), and valine (V). Very small amino acids are alanine (A), glycine (G) and serine (S). Amino acids likely to be involved in a turn formation are alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), glycine (G), histidine (H), lysine (K),
30 asparagine (N), glutamine (Q), arginine (R), serine (S), proline (P), and threonine (T).

reported to be important for binding and activity, this invention surprisingly provides amino acid sequences which define common binding motifs on IR and IGF-1R which are capable of conferring agonist and/or antagonist activity at these receptors. In addition, this invention identifies multiple
5 binding sites (Sites 1 and 2) on IR and IGF-1R which appear to be allosterically coupled.

Although capable of binding to IR and/or IGF-1R at sites which participate in conferring agonist or antagonist activity, the amino acid sequences are neither based on insulin or IGF-1 native sequences, nor do
10 they reflect an obvious homology to any such sequence.

The amino acid sequences of the invention may be peptides, polypeptides, or proteins. These terms as used herein should not be considered limiting with respect to the size of the various amino acid sequences referred to herein and which are encompassed within this
15 invention. Thus, any amino acid sequence comprising at least one of the IR or IGF-1R binding motifs disclosed herein, and which binds to one of the receptors is within the scope of this invention. In preferred embodiments, the amino acid sequences confer insulin or IGF agonist or antagonist activity. The amino acid sequences of the invention are typically artificial,
20 i.e. non-naturally occurring peptides or polypeptides. Amino acid sequences useful in the invention may be obtained through various means such as chemical synthesis, phage display, cleavage of proteins or polypeptides into fragments, or by any means which amino acid sequences of sufficient length to possess binding ability may be made or obtained.

25 The amino acid sequences provided by this invention should have an affinity for IR or IGF-1R sufficient to provide adequate binding for the intended purpose. Thus, for use as a therapeutic, the peptide, polypeptide or protein provided by this invention should have an affinity (K_d) of between about 10^{-7} to about 10^{-15} M. More preferably the affinity is 10^{-8} to about
30 10^{-12} M. Most preferably, the affinity is 10^{-9} to about 10^{-11} M. For use as a reagent in a competitive binding assay to identify other ligands, the amino

Figure 69: Competition of MBP-peptide fusion proteins to rVab 12H10 binding to insulin receptor. Four MBP-peptides fusion proteins at indicated concentrations were incubated with rVab 12H10 overnight using the heterogeneous TRFA.

5 Figures 70A-70N: Peptide binding displacement curves showing the displacement of 125 I-insulin or 125 I-IGF-1 from HIR or HIGF-1R in the presence of various peptides.

Figures 71A-71Z; 71A2-71Z2; 71A3-71B3: Concentration dependent modulation of 3 H-glucose into adipocytes by various peptides. Formula 1
10 motif peptide responses are shown in Figures 71A-71V; 71A2-71J2; Formula 9 motif peptide response is shown in Figures 71W-71Z; Formula 2 motif peptide response is shown in Figures 71K2-71L2; Miscellaneous peptide motif 10 peptide responses are shown in Figures 71M2-71P2; Formula 6 motif peptide response is shown in Figure 71Q2-Figure 71R2;
15 and Formula 4 motif peptide response is shown in Figure 71S2-Figure 71W2. Formula 1 and Formula 2 motif peptide response is shown in Figure 71X2-Figure 71A3. Fusion peptide S291 response is shown in Figure 71B3.

Figures 72A and 72B: Competition of Site 1(Figure 72B) and Site 2 (Figure 72A) phage displayed peptides with recombinant cleaved dipeptides.

20 Figure 73: Competition of IGF-1R, peptide H2C (D117), peptide C1 (D112), and peptide RP6 (20C-3-G3-IGFR) in a homogeneous fluorescent-resonance energy transfer assay based on the binding of IGF-1R to peptide 20E2 (D118).

Figure 74: Stimulation of IR autophosphorylation *in vivo* by MBP-
25 fusion peptides.

V. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to amino acid sequences comprising motifs which bind to the IGF-1 receptor (IGF-1R) and/or the insulin receptor (IR). In addition to binding to IR and-IGF-1R; the amino acid sequences also
30 possess either agonist, partial agonist or antagonist activity at one or both of these receptors. Based on the differing regions of IR and IGF-1R which are

containing cysteine residues. The hatched bars indicate value is $\leq 54\%$ control value. Figure 54B. Competition with fusion proteins containing the consensus sequence. The notation, c-c, indicates phage displayed peptides with cysteine residues. Figure 54C. Competition with fusion protein
5 containing a control peptide.

Figure 57: Nucleotide and predicted amino acid sequence of the gene encoding the 6f6 rVab that binds to IR.

Figure 58: Nucleotide and predicted amino acid sequence of the gene encoding the 14c8 rVab that binds to IR.

10 Figure 59: Comparison of the VH CDR3 sequences of different rVabs that bind to IR, and competitions of these rVabs and insulin for binding to IR.

Figure 60: Biological response of insulin, rVab 12h10, and rVab 13h9 in 32D cells expressing or not expressing IR.

15 Figure 61: Competition of rVab 6f6 and insulin for binding to IR.

Figure 62: Competition of rVab 6f6 and IGF-1 for binding to IR.

Figure 63: Competition of synthetic peptides and soluble rVab antibodies for binding of biotinylated peptides to insulin receptor. Synthetic peptides or soluble rVab at indicated concentrations were incubated with
20 biotinylated peptides overnight using the heterogeneous TRFA.

Figure 64: Binding of C1 to IR and IGF-1R.

Figure 65: Competition of peptides for binding to IR.

Figure 66: H2C competition for b-peptide binding to IR. Biotinylated peptides at indicated concentrations were competed by increasing
25 concentrations of H2C for binding to IR using the heterogeneous TRFA.

Figure 67: C1C competition for b-C1 binding to IR. Biotinylated C1 peptide at $0.3\ \mu\text{M}$ was competed by increasing concentrations of C1C for binding to IR using the heterogeneous TRFA.

Figure 68: Competition of peptides for binding of rVab 12H10 to
30 insulin receptor. Synthetic peptides at indicated concentration were incubated with rVab 12H10 overnight using the heterogeneous TRFA.

Figure 48: Biopanning results and sequence alignments of Groups 2 through 7 of IR-binding peptides. The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor.

5 Figure 49A-49D: Dose response curve of D118 peptide (Formula 2 motif) stimulated increase of ^3H -glucose into mouse adipocytes.

Figures 50A-50D: Titration of the synthetic peptides C1 (Figures 50A, 50C) or B6 (Figures 50B, 50D) against constant concentration of phage bound to IR (Figures 50A, 50B) or IGF-1R (Figures 50C, 50D).

10 Phage are represented by: open circle – 20D3; open square – 20A4; open triangle – 20E2; open diamond – F2; filled circle – F8; and filled square – D8.

Figure 51A-51D: Titration of the IGF-1R synthetic peptides against constant concentration of phage. Symbols for the peptides are: open circles
15 – H2; filled circles – H2C; open square – C1; filled square – C1C; open triangle – D2C; filled triangle – E4; open diamond – A6; and filled diamond p53.

Figure 52A-52D: Hill plot analysis of phage clones. The detailed data are provided in Table 7. Symbols are the same as in Figure 51.

20 Figure 53: Competition between the insulin and the IR-binding phage. The results for seven different groups (categories) of phage binders are shown.

Figure 54: Titration of the synthetic peptide 20A4 against constant concentration of phage. Phage binding to IR are represented by: open
25 circle – 20D3; filled circle B8; open square – 20A4; filled square – D8; open up triangle – 20E2; open down triangle – D10; filled down triangle – A2; open diamond – F2; filled diamond – E8; and cross-filled circle – F8.

Figure 55: A schematic drawing for the construction of protein fusions of the maltose binding protein and peptides from phage libraries.

30 Figure 56A-56C: Insulin Receptor Competition ELISA using MBP-Peptide Fusion Proteins. Figure 54A. Competition with fusion proteins

Figure 37: Nucleotide sequence of gene encoding the M100 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

5 Figure 38: Nucleotide sequence of gene encoding the 46A7 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 39: Nucleotide sequence of gene encoding the 49E8 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

10 Figure 40: Assay results demonstrating the binding of soluble forms of three rVabs to IGF-1R.

Figure 41: Assay results showing that the 43G7 rVab stimulates growth of IGF-1R⁺ cells.

15 Figure 42: Assay results showing that the stimulation by rVab 43G7 is antagonized by the 1G2P, 49E8, and 46A7 rVabs. The assay was done on IGF-1R⁺ cells.

Figure 43: Eu-based fluorescence assay results showing that the binding of peptide 5.1 to IGF-1R can be competed by the IGF-1 ligand.

20 Figure 44: Results of the time-resolved fluorescence assay showing that the binding of 43G7 rVab to IGF-1R is effectively competed by IGF-1.

Figure 45: Eu-based fluorescence assay showing that the binding of the B6 peptide to IGF-1R is effectively competed by the 43G7 rVab.

25 Figures 46A-46D: Results of the Eu-based fluorescence assay showing that the binding of the europium-labeled 43G7 rVab to IGF-1R is effectively competed by selected scAbs specific for IGF-1R.

Figure 47: Biopanning results and sequence alignments of Group 1 of IR-binding peptides. The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: +, +, +, +, >30X over background; +, +, +, 15-30X; +, +, 5-15X; +, 2-5X; and 0, <2X.

30

Figures 26A and 26B: Design of the secondary phage library A6S based on the Motif 1 peptide sequences. Figure 26A: Sequence design for the A6S secondary phage library. Figure 26B: Synthetic oligonucleotide for the A6L secondary library. Definitions of mixes (all mixes are equimolar) are as follows: N = A, C, G, or T; K = G or T. Nucleosides were premixed in the bottle (and not line mixed) to improve the accuracy of nucleoside mixes. The sequence of the FLAG epitope is shown in bold.

Figure 27: Sequences of the five H5-like peptides that show agonistic activity toward IGF-1R. The C-terminal lysine contains a biotin moiety linked to the amino group of the side chain.

Figure 28: Listing of amino acid sequences obtained from panning with the A6S library.

Figure 29: Listing of amino acid sequences obtained from panning with the H5 secondary phage library.

Figure 30: Schematic of the genomic rVab library.

Figure 31: Listing of the V_H, kappa and lambda genes used to assemble the rVab antibody library for IGF-1R binders.

Figure 32: Schematic of the assembly of the single-chain IGF-I and insulin antibody libraries from restriction fragments.

Figure 33: Sequences of the restriction fragments used to assemble the rVab libraries.

Figure 34: Nucleotide sequence of the gene encoding the 43G7 rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 35: Nucleotide sequence of the gene encoding the 1G2P rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 36: Nucleotide sequence of gene encoding the 39F7 rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 19: DNA sequences of the frameshifted clones.

Figures 20A and 20B: Results of the phage ELISA for binding to IGF-1R. Wells were coated with 100 ng/well IGF-1R and blocked. Competitor, the IGF-1 native ligand, was present prior (1 h) and during the phage incubation (1 h). Phage were detected with HRP-anti M13 phage antibody and reported as OD₄₀₅ as described. Total Binding is shown in Figure 20A and Percent Inhibition is shown in Figure 20B.

Figure 21: Sequences of the designed IGF-1R-specific synthetic peptides.

Figure 22: Assay results showing that Motif 2 peptides (5.1 and 5.2) antagonize the effects of IGF-1 on IGF-1R⁺ cells.

Figure 23: Assay results showing that Motif 1 peptides (5.3 and 5.4) stimulate growth of IGF-1R⁺ cells. Cells expressing human IGF-1R (30,000 cells per well) were incubated with the 5.4 peptide for 42 h at 37°C.

Experiments were done in triplicate. Background signal A₄₅₀=0.15. Proliferation was measured using WST-1 reagent (Boehringer Mannheim Biochemicals/Roche Molecular Biochemicals, Indianapolis, IN).

Figures 24A and 24B: Demonstration of binding of peptide 5.1 to IGF-1R using BIAcore. Figure 24A: Binding as a function of the peptide concentration. Figure 24B: Inhibition of IGF-1 binding by peptide 5.1. RU – refractive units.

Figures 25A and 25B: Design of the secondary phage library A6L based on the Class II peptide sequences. Figure 25A: Design of the sequence of the gene. Underlined residues indicate positions mutated to optimize the codons for expression in *E. coli*. Figure 25B: Synthetic oligonucleotide for the A6L secondary library. Underlined residues were doped in the chemical DNA synthesis. Definitions of mixes (all mixes are equimolar) are as follows: N = A, C, G, or T; K = G or T. Nucleosides were premixed in the bottle (and not line mixed) to improve the accuracy of nucleoside mixes. The sequence of the FLAG epitope is shown in bold.

Figure 10G: Miscellaneous peptide sequences identified from a library constructed to contain variations of the F8 peptide as indicated (F815) panned against IGF-1R.

5 Figure 10H: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide as indicated (referred to as "NNKH") panned against IR.

Figure 10I: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide as indicated (referred to as "NNKH") panned against IGF-1R.

10 Figure 11A: Summary of specific representative amino acid sequences from Formulas 1 through 11.

Figure 11B: Summary of specific representative amino acid sequences from Formulas 1 through 11.

15 Figure 12: Illustration of helix wheels applied to Formula 2 and 3 motifs.

Figure 13: Illustration of 2 binding site domains on IR based on competition data.

20 Figure 14: Dissociation of 20E2 peptide from IGF-1R in the presence of buffer (filled circle), 30 μ M IGF-1 (open circle), 100 μ M H2C (filled square), 100 μ M 20E2 (filled triangle), 100 μ M D8 (B12; open square), 100 μ M C1 (filled, inverted triangle) and 100 μ M RPG (filled diamond).

Figure 15: Schematic illustration of potential binding schemes to the multiple binding sites on IR.

25 Figure 16: Schematic diagram of the phage-displayed peptide library. The peptide is displayed as a protein fusion to the N-terminus of gene III encoding the minor coat protein of the phage.

Figure 17: BIAcore analysis of competition binding between IR and MBP fusion H2C-9-H2C, H2C and H2C-3-H2C.

30 Figure 18: Sequence alignments of Class I and Class II peptides. The Class I peptides have been shown to be IGF-1R antagonists, while the Class II peptides are IGF-1R agonists.

Figure 6E: Formula 6 motif peptide sequences identified from other libraries panned against IR.

Figure 7: Formula 7 motif peptide sequences.

Figure 8: Formula 8 motif peptide sequences identified from a
5 commercial phage display peptide library and synthetic sequences. Small letters denote D-amino acids. Unnatural amino acids are denoted with a 3-letter abbreviation in certain sequences. K_d values greater than 2×10^{-5} are approximate.

Figure 9A: Formula 9 motif peptide sequences identified from a
10 library constructed to contain variations in the H5 peptide as indicated (referred to as "H5") panned against IGF-1R.

Figure 9B: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the JBA5 peptide as indicated (referred to as "JBA5") panned against IGF-1R.

15 Figure 9C: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the JBA5 peptide as indicated (referred to as "JBA5") panned against IR.

Figure 10A: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IGF-1R.

20 Figure 10B: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IR.

Figure 10C: Miscellaneous peptide sequences identified from a random 20mer library panned against IR.

25 Figure 10D: Miscellaneous peptide sequences identified from a random 40mer library panned against IR.

Figure 10E: Miscellaneous peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 10F: Miscellaneous peptide sequences identified from a $X_{1-4}CX_{6-20}$ and panned against IGF-1R.

Figure 3D: Formula 3 motif peptide sequences identified from a library constructed using the sequence $X_3LXXLXXYFX_{12-17}$ (reverse B6; rB6) panned against IGF-1R.

5 Figure 4A: Formula 4 motif peptide sequences identified from a random 20mer library panned against IR.

Figure 4B: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IR.

10 Figure 4C: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IGF-1R.

Figure 4D: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (20% dope; referred to as "F820") panned against IR.

15 Figure 4E: Formula 4 motif peptide sequences identified from other libraries panned against IR.

Figure 5: Formula 5 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IGF-1R.

20 Figure 6A: Formula 6 motif peptide sequences identified from a random 20mer library and panned against IR.

Figure 6B: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (15% dope; referred to as "D815") panned against IR.

25 Figure 6C: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (20% dope; referred to as "D820") panned against IR.

30 Figure 6D: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (20% dope; referred to as "D820") panned against IGF-1R.

Figure 2I: Formula 2 motif peptide sequences identified from a library constructed to contain a helix-turn-helix based on the B6 peptide as indicated (referred to as "B6H") and panned against IGF-1R.

Figure 2J: Formula 2 motif peptide sequences identified from a library constructed to contain variations in the consensus core of B6 peptide as indicated (referred to as "B6C") and panned against IR.

Figure 2K: Formula 2 motif peptide sequences identified from a library constructed to contain variations in the consensus core of B6 peptide as indicated (referred to as "B6C") and panned against IGF-1R.

Figure 2L: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYDAIDQLVX}_{16-21}$ (20E2-A) panned against IR.

Figure 2M: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYDAIDQLVX}_{16-21}$ (20E2-A) panned against IGF-1R.

Figure 2N: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYXXhXXhhX}_{16-21}$ (20E2-B) panned against IR.

Figure 2O: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYXXhXXhhX}_{16-21}$ (20E2-B) panned against IGF-1R.

Figure 2P: Formula 2 motif peptide sequences identified from a library constructed using the sequence $X_{1-6}\text{FYRYFXXLLX}_{16-21}$ (NNRP) panned against IR.

Figure 3A: Formula 3 motif peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 3B: Formula 3 motif peptide sequences identified from a $X_{1-4}\text{CX}_{6-20}$ library panned against IGF-1R.

Figure 3C: Formula 3 motif peptide sequences identified from a library constructed using the sequence $X_3\text{LXXLXXYFX}_{12-17}$ (reverse B6; rB6) panned against IR.

Figure 1L: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6} FHENFYDWFVRQVSX X_{21-26} (H2C-A) panned against IGF-1R.

Figure 1M: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6} FHXXFYXWFX X_{16-21} (H2C-B) and panned against IR.

Figure 1N: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6} FHXXFYXWFX X_{16-21} (H2C-B) and panned against IGF-1R.

Figure 1O: Formula 1 motif peptide sequences obtained from other libraries panned against IR.

Figure 2A: Formula 2 motif peptide sequence identified from a random 40mer library panned against IR.

Figure 2B: Formula 2 motif peptide sequences identified from a random 40mer library panned against IGF-1R.

Figure 2C: Formula 2 motif peptide sequences identified from a random 20mer library panned against IR.

Figure 2D: Formula 2 motif peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 2E: Formula 2 motif peptide sequences identified from a $X_{1-4}CX_{6-20}$ library panned against IGF-1R.

Figure 2F: Formula 2 motif peptide sequences identified from a library constructed to contain variations outside the consensus core of the B6 peptide as indicated (referred to as "B6L") and panned against IR.

Figure 2G: Formula 2 motif peptide sequences identified from a library constructed to contain variations outside the consensus core of the B6 peptide as indicated (referred to as "B6L") and panned against IGF-1R.

Figure 2H: Formula 2 motif peptide sequences identified from a library constructed to contain a helix-turn-helix based on the B6 peptide as indicated (referred to as "B6H") and panned against IR.

Figure 1A: Formula 1 motif peptide sequences obtained from a random 40mer library panned against IR.

Figure 1B: Formula 1 motif peptide sequence obtained from a random 40mer library panned against IGF-1R.

5 Figure 1C: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IR.

Figure 1D: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IGF-1R.

Figure 1E: Formula 1 motif peptide sequences obtained from a
10 21mer library constructed to contain X_{1-10} NFYDWFVX $_{18-21}$ (also referred to as "A6S") panned against IR.

Figure 1F: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X_{1-10} NFYDWFVX $_{18-21}$ (also referred to as "A6S") panned against IGF-1R.

15 Figure 1G: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L") panned against IR.

Figure 1H: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the
20 A6 peptide as indicated (referred to as "A6L") panned against IGF-1R.

Figure 1I: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (as indicated) panned against IR.

Figure 1J: Formula 1 motif peptide sequences obtained from a
25 library constructed to contain variations in the consensus core of the E4D peptide (as indicated) panned against IGF-1R.

Figure 1K: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6} FHENFYDWFVRQVSX $_{21-26}$ (H2C-A) panned against IR.

therapeutics themselves or may be used to identify other molecules, especially small organic molecules, which possess agonist or antagonist activity at IR or IGF-1R.

- Another object of this invention is to provide structural information
5 derived from the amino acid sequences of this invention which may be used to construct other molecules possessing the desired activity at the relevant IR of IGF-1R binding site.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

- Figures 1A-10G. Amino acid sequences comprising the motif of
10 Formulas 1 through 10. Sequences were identified by panning peptide libraries against IGF-1R and/or IR. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR Ratio Comparison is determined by dividing
15 the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R Ratio Comparison is determined by dividing the ratio of IR by the ratio of IGF-1R.

- The design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are
20 held constant. Additional abbreviations in the B6H library are: 'O' indicates an NGY codon where Y is C or T; 'J' indicates an RHR codon where R is A or G, and H is A, C, or T; and 'U' indicates an VVY codon where V is A, C, or G, and Y is C or T. The 'h' in the 20E2 libraries indicates an NTN codon.

- Symbols in the listed sequences are: Q - TAG Stop; # -TAA Stop; * -
25 TGA Stop; and ? - Unknown Amino Acid. It is believed that a W replaces the TGA Stop Codon when expressed. Except for the 20C, A6L, and B6L libraries, all libraries are designed with the short FLAG Epitope DYKD (Hopp *et al.*, 1988) at the N-terminus of the listed sequence and AAAGAP at the C-terminus. The 20C, A6L, and B6L libraries have the full length FLAG
30 epitope DYKDDDDDK.

different sites on one receptor or to cause receptor complexing through different sites.

The present invention also provides assays for identifying compounds that mimic the binding characteristics of insulin. Such compounds may act
5 as antagonists or agonists of insulin function in cell based assays.

This invention also provides amino acid sequences such as peptides and recombinant antibody variable regions (rVab) that inhibit binding of insulin to the insulin receptor. Such amino acid sequences and rVabs are used in the assays of the invention to identify compounds that mimic insulin.

10 This invention also provides kits for identifying compounds that bind to the insulin receptor. The invention further provides therapeutic compounds that bind the insulin receptor.

In another embodiment, this invention provides assays for identifying compounds which mimic the binding characteristics of IGF-1. Such
15 compounds act as antagonists or agonists of IGF-1 hormone function in cell based assays.

The invention also provides amino acid sequences such as peptides and rVabs which inhibit binding of IGF-1 to IGF-1R. Such amino acid sequences and rVabs are used in the assays of the invention to identify
20 compounds which mimic IGF-1.

Another embodiment of this invention is the nucleic acid sequences encoding the amino acid sequences of the invention. Also within the scope of this invention are vectors containing the nucleic acids and host cells which express the genes encoding the amino acid sequences which bind at
25 IR or IGF-1R and possess agonist or antagonist activity.

It is an object of this invention to provide amino acid sequences which bind to active sites of IR and/or IGF-1R and to identify structural criteria for conferring agonist or antagonist activity at IR and/or IGF-1R.

It is a further object of this invention to provide specific amino acid
30 sequences which possess agonist, partial agonist or antagonist activity at either IR or IGF-1R. Such amino acid sequences are potentially useful as

i. an amino acid sequence comprising the sequence
DYKDLCSWGVRIGWLAGLCPKK.

j. $WX_{123} GYX_{124} WX_{125} X_{126}$ wherein X_{123} is selected from
proline, glycine, serine, arginine, alanine or leucine, but more preferably
5 proline; X_{124} is any amino acid, but preferably a charged or aromatic amino
acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine,
and most preferably leucine. X_{126} is any amino acid, but preferably a small
amino acid.

In one embodiment, preferred amino acid sequences $FYX_3 WF$ ("A6"
10 motif) and $FYX_8 X_9 L/IX_{11} X_{12} L$ ("B6" motif) have been identified which
competitively bind to sites on IR and IGF-1R and possess either agonist or
antagonist activity. Surprisingly $FYX_3 WF$ which possesses agonist activity
at IGF-1R, can possess agonist or antagonist activity at IR. Similarly, $FY X_8$
 $X_9 L/IX_{11} X_{12} L$, which is an antagonist at IGF-1R, possesses agonist activity
15 at IR.

This invention also identifies at least two distinct binding sites on IR
and IGF-1R based on the differing ability of certain of the peptides to
compete with one another and insulin or IGF-1 for binding to IR and IGF-1R.
Accordingly, this invention provides amino acid sequences which bind
20 specifically to one or both sites of IR and/or IGF-1R. Furthermore, specific
amino acid sequences are provided which have either agonist or antagonist
characteristics based on their ability to bind to the specific sites of IR.

In another embodiment of this invention, amino acid sequences which
bind to one or more sites of IR or IGF-1R may be covalently linked together
25 to form multivalent ligands. These multivalent ligands are capable of
forming complexes with a plurality of IR or IGF-1R. Either the same or
different amino acid sequences may be covalently bound together to form
homo- or heterocomplexes. Dimers of the same amino acid sequence, for
example, may be used to form receptor complexes bound through the same
30 corresponding sites. Alternatively, heterodimers may be used to bind to

c. $X_{14} X_{15} X_{16} X_{17} X_{18} X_{19} X_{20} X_{21}$ wherein X_{14} , and X_{17} are hydrophobic amino acids, X_{15} , X_{16} , X_{18} and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

d. $X_{22} X_{23} X_{24} X_{25} X_{26} X_{27} X_{28} X_{29} X_{30} X_{31} X_{32} X_{33} X_{34} X_{35} X_{36}$
 5 $X_{37} X_{38} X_{39} X_{40} X_{41}$ wherein X_{22} , X_{25} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{36} , X_{37} , X_{38} , X_{40} , and X_{41} are any amino acid, X_{35} and X_{37} may be any amino acid for binding to IR, whereas X_{35} is preferably a hydrophobic amino acid and X_{37} is preferably glycine for binding to IGF-1R and possess agonist or antagonist activity. X_{23} and X_{26} are hydrophobic amino acids. This sequence further
 10 comprises at least two cysteine residues, preferably at X_{25} and X_{40} X_{31} and X_{32} are small amino acids.

e. $X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} X_{56}$
 $X_{57} X_{58} X_{59} X_{60} X_{61}$ wherein X_{42} , X_{43} , X_{44} , X_{45} , X_{53} , X_{55} , X_{56} , X_{58} , X_{60} and X_{61} may be any amino acid, X_{43} , X_{46} , X_{49} , X_{50} , X_{54} are hydrophobic amino acids,
 15 X_{47} and X_{59} are preferably cysteines, X_{48} is a polar amino acid, and X_{51} , X_{52} and X_{57} are small amino acids.

f. $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76}$
 $X_{77} X_{78} X_{79} X_{80} X_{81}$ wherein X_{62} , X_{65} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} , and X_{81} may be any amino acid; X_{63} , X_{70} , X_{74} are hydrophobic amino acids; X_{64} is
 20 a polar amino acid, X_{67} and X_{75} are aromatic amino acids and X_{72} and X_{79} are preferably cysteines capable of forming a loop.

g. $H X_{82} X_{83} X_{84} X_{85} X_{86} X_{87} X_{88} X_{89} X_{90} X_{91} X_{92}$ wherein X_{82} is proline or alanine, X_{83} is a small amino acid, X_{84} is selected from leucine, serine or threonine, X_{85} is a polar amino acid, X_{86} , X_{88} , X_{89} and X_{90} are any
 25 amino acid, and X_{87} , X_{91} and X_{92} are an aliphatic amino acid.

h. $X_{104} X_{105} X_{106} X_{107} X_{108} X_{109} X_{110} X_{111} X_{112} X_{113} X_{114}$
 wherein at least one of the amino acids of X_{106} through X_{111} , and preferably two, are tryptophan separated by three amino acids, and wherein at least one of X_{104} , X_{105} and X_{106} and at least one of X_{112} , X_{113} and X_{114} are
 30 cysteine; and

III. SUMMARY OF THE INVENTION

This invention relates to the identification of amino acid sequences that specifically recognize sites involved in IR and/or IGF-1R activation. Specific amino acid sequences are identified and their agonist or antagonist activity at IR or IGF-1R has been determined. Such sequences may be developed as potential therapeutics or as lead compounds to develop other more efficacious ones. In addition, these sequences may be used in high-throughput screens to identify and provide information on small molecules which bind at these sites and mimic or antagonize the functions of insulin or IGF-1. Furthermore, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

In one aspect of this invention large numbers of peptides have been screened for their IR or IGF-1R binding and activity characteristics. Analysis of their amino acid sequences has identified certain consensus sequences which may be used themselves or as core sequences in larger amino acid sequences conferring upon them agonist or antagonist activity. At least ten generic amino acid sequences have been identified which bind IR and IGF-1R with varying degrees of agonist or antagonist activity depending on the specific sequence of the various peptides identified within each motif group. Also provided are amino or carboxyl terminal extensions capable of modifying the affinity and/or pharmacological activity of the consensus sequences when part of a larger amino acid sequence.

The amino acid sequences of this invention which bind IR and/or IGF-1R include:

- a. $X_1 X_2 X_3 X_4 X_5$ wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, and X_3 is any polar amino acid;
- b. $X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13}$ wherein X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} and X_{12} are any amino acid, and X_{10} and X_{13} are hydrophobic amino acids;

desirable to identify other molecular structures which mimic the active binding regions of insulin and/or IGF-1 and which impart selective agonist or antagonist activity.

Although certain proteins are important drugs, their use as
5 therapeutics presents several difficult problems, including the high cost of production and formulation, administration usually via injection and limited stability in the bloodstream. Therefore, replacing proteins, including insulin or IGF-1, with small molecular weight drugs has received much attention. However, none of these efforts has resulted in finding a successful drug.

10 Peptides mimicking functions of protein hormones have been previously reported. Yanofsky *et al.* (1996) reports the isolation of a monomer peptide antagonistic to IL-1 with nanomolar affinity for the IL-1 receptor. This effort required construction and use of many phage displayed peptide libraries and sophisticated phage panning procedures.

15 Wrighton *et al.* (1996) and Livnah *et al.* (1996) reported dimer peptides that bind to the erythropoietin (EPO) receptor with full agonistic activity *in vivo*. These peptides are cyclical and have intra-peptide disulfide bonds; like the IL-1 receptor antagonist, they show no significant sequence identity to the natural ligand. Importantly, X-ray crystallography revealed
20 that it was the spontaneous formation of non-covalent peptide homodimers which enabled the dimerization two EPO receptors.

Most recently, Cwirla *et al.* (1997) reported the identification of two families of peptides that bind to the human thrombopoietin (TPO) receptor and are competed by the binding of the natural TPO ligand. The peptide
25 with the highest affinity, when dimerized by chemical means proved to be as potent an *in vivo* agonist as TPO, the natural ligand .

WO 96/04557 reports the use of peptides and antibodies which bind to active sites of biological targets and which are then used in competition assays to identify small molecules which are agonist or antagonists at the
30 biological targets.

Both the N-terminal and C-terminal regions (located within the putative L1 and L2 domains) of the IR are important for high-affinity insulin binding but appear to have little effect on IGF-1 binding. Replacing residues in the N-terminus of IGF-1R (amino acids 1-62) with the corresponding residues of IR (amino acids 1-68) confers insulin-binding ability on IGF-1R. Within this region residues Phe-39, Arg-41 and Pro-42 are reported as major contributors to the interaction with insulin. (Williams *et al.*, 1995). When these residues are introduced into the equivalent site of the IGF-1R, the affinity for insulin is markedly increased, whereas, substitution of these residues by alanine in the IR results in markedly decreased insulin affinity. Similarly, the region between amino acids 704-717 of the C-terminus of IR has been shown to play a major role in insulin specificity. Substitution of these residues with alanine also disrupts insulin binding. (Mynarcik *et al.*, 1996).

Further studies of alanine scanning of the receptors suggest that insulin and IGF-1 may use some common contacts to bind to the IGF-1 receptor but that those contacts differ from those that insulin utilizes to bind to the insulin receptor. (Mynarcik *et al.*, 1997). Hence, the data in the literature has led one commentator to state that even though "the binding interfaces for insulin and IGF-1 on their respective receptors may be homologous within this interface the side chains which make actual contact and determine specificity may be quite different between the two ligand-receptor systems." (De Meyts, 1994).

The identification of molecular structures having a high degree of specificity for one or the other receptor is important to developing efficacious and safe therapeutics. For example, a molecule developed as an insulin agonist should have little or no IGF-1 activity in order to avoid the mitogenic activity of IGF-1 and a potential for facilitating neoplastic growth.

It is therefore important to determine whether insulin and IGF-1 share common three-dimensional structures but which have sufficient differences to confer selectivity for their respective receptors. Similarly, it would be

making the mature IGF-1 slightly larger than insulin. (Blakesley, 1996). The C region of human insulin-like growth factor (IGF-1) appears to be required for high affinity binding to the type I IGF receptor. (Pietrzkowski *et al.*, 1992). Specifically, tyrosine 31 located within this region appears to be essential for high affinity binding. Furthermore, deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the IR, while decreasing its affinity for the IGF-1R receptor. (Pietrzkowski *et al.*, 1992). A further structural distinction between the two hormones is that, unlike insulin, IGF-1 has very weak self-association and does not hexamerize. (De Meyts, 1994).

The α -subunits, which contain the ligand binding region of the IR and IGF-1R, demonstrate between 47-67% overall amino acid homology. Three general domains have been reported for both receptors from sequence analysis of the α subunits, L1-Cys-rich-L2. The cysteine residues in the C-rich region are highly conserved between the two receptors; however, the cysteine-rich domains have only 48% overall amino acid homology.

Despite the similarities observed between these two receptors, the role of the domains in specific ligand binding are distinct. Through chimeric receptor studies, (domain swapping of the IR and IGF-1R α -subunits), researchers have reported that the sites of interaction of the ligands with their specific receptors differ. (Blakesley, *et al.*, 1996). For example, the cysteine-rich domain of the IGF-1R (amino acids 191-290) was determined to be essential for high-affinity IGF binding, but not insulin binding by introducing this IGF-1R region into the corresponding region of the IR (amino acids 198-300) and observing that the IR demonstrated high affinity binding of IGF-1 while maintaining high-affinity insulin binding. Conversely, when the corresponding region of the IR was introduced into the IGF-1R, the affinity for IGF-1 was not detectable while the affinity for insulin remained undisturbed.

A further distinction between the binding regions of the IR and IGF-1R is their differing dependence on the N-terminal and C-terminal regions.

be similar. The crystal structure of the first three domains of IGF-1R has been determined (Garrett *et al.*, 1998). The L domains consist of a single-stranded right-handed β -helix (a helical arrangement of β -strands), while the cysteine-rich region is composed of eight disulfide-bonded modules.

5 The β -subunit of the insulin receptor has 620 amino acid residues and three domains: extracellular, transmembrane, and cytosolic. The extracellular domain is linked by disulfide bridges to the α -subunit. The cytosolic domain includes the tyrosine kinase domain, the three-dimensional structure of which has been solved (Hubbard *et al.*, 1994).

10 To aid in drug discovery efforts, a soluble form of a membrane-bound receptor was constructed by replacing the transmembrane domain and the intracellular domain of IR with constant domains from immunoglobulin Fc or λ subunits (Bass *et al.*, 1996). The recombinant gene was expressed in human embryonic kidney 293 cells. The expressed protein was a fully
15 processed heterotetramer and the ability to bind insulin was similar to that of the full-length holoreceptor.

 IGF-1 and insulin competitively cross-react with IGF-1R and IR. (Schäffer, 1994). Despite 45% overall amino acid homology, insulin and IGF-1 bind only weakly to each other's receptor. The affinity of each peptide
20 for the non-cognate receptor is about 3 orders of magnitude lower than that for the cognate receptor. (Mynarcik, *et al.*, 1997). The differences in binding affinities may be partly explained by the differences in amino acids and unique domains which contribute to unique tertiary structures of ligands. (Blakesley *et al.*, 1996).

25 Both insulin and IGF-1 are expressed as precursor proteins comprising, among other regions, contiguous A, B, and C peptide regions, with the C peptide being an intervening peptide connecting the A and B peptides. A mature insulin molecule is composed of the A and B chains connected by disulfide bonds, whereas the connecting C peptide has been
30 removed during post-translational processing. IGF-1 retains its smaller C-peptide as well as a small D extension at the C-terminal end of the A chain,

composed of two α and two β subunits which form a disulfide-linked heterotetramer (β - α - α - β). They have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity. The extracellular domain is composed of the entire
5 subunits and a portion of the N-terminus of the β subunits, while the intracellular portion of the β subunits contains the tyrosine kinase domain. Besides IR and IGF-1R, the other known member of the IR family is the insulin-related receptor (IRR), for which no natural ligand is known.

While similar in structure, IGF-1 and insulin receptors serve different
10 physiological functions. The IR is primarily involved in metabolic functions whereas the IGF-1R mediates growth and differentiation. However, both insulin and IGF-1 can induce both mitogenic and metabolic effects. Whether each ligand elicits both activities via its own receptor, or whether insulin exerts its mitogenic effects through its weak affinity binding to the IGF-1
15 receptor, and IGF-1 its metabolic effects through the insulin receptor, remains controversial. (De Meyts, 1994).

The insulin receptor is a glycoprotein having molecular weight of 350-400 kDa (depending of the level of glycosylation). It is synthesized as a single polypeptide chain and proteolytically cleaved yielding the disulfide-
20 linked monomer α - β insulin receptor. Two α - β monomers are linked by disulfide bonds between the α -subunits to form a dimeric form of the receptor (β - α - α - β -type configuration). The α subunit is comprised of 723 amino acids, and it can be divided into two large homologous domains, L1 (amino acids 1-155) and L2 (amino acids 313-468), separated by a cysteine
25 rich region (amino acids 156-312) (Ward *et al.*, 1995). Many determinants of insulin binding seem to reside in the α -subunit. A unique feature of the insulin receptor is that it is dimeric in the absence of ligand.

The sequence of IR is highly homologous to the sequence of the type-1 insulin-like growth factor receptor (IGF-1R). The homology level
30 varies from about 40% to 70%, depending on the position within the α -subunit. The three-dimensional structures of both receptors may therefore

repair, and recently has been implicated in various forms cancer including prostate, breast, colorectal, and ovarian. It is similar in size, sequence and structure to insulin, but has 100-1,000-fold lower affinity for the insulin receptor (Mynarcik *et al.*, 1997).

5 Clinically, recombinant human IGF-1 has been investigated for the treatment of several diseases, including type I diabetes (Carroll *et al.*, 1997; Crowne *et al.*, 1998), amyotrophic lateral sclerosis (Lai *et al.*, 1997), and diabetic motor neuropathy (Apfel and Kessler, 1996). Other potential therapeutic applications of IGF-1 such as osteoporosis (Canalis, 1997),
10 immune modulation (Clark, 1997) and nephrotic syndrome (Feld and Hirshberg, 1996) are being examined.

 A number of studies have analyzed the role of natural IGF-1 in various disease states. Most interestingly, several reports have shown that IGF-1 promotes the growth of normal and cancerous prostate cells both *in*
15 *vitro* and *in vivo* (Angelloz-Nicoud and Binoux, 1995; Figueroa *et al.*, 1995; Torring *et al.*, 1997). Additionally, elevated serum IGF-1 levels have been connected with increased risks of prostate cancer, and may be an earlier predictor of cancer than is prostate-specific antigen (PSA) (Chan *et al.*, 1998). Recent studies have indicated a connection between IGF-1 and
20 other cancers such as breast, colorectal, and ovarian. Serum IGF-1 levels are regulated by the presence of IGF binding proteins (IGFBP) which bind to IGF-1 and prevent its interaction with the IGF-1R (reviewed in Conover, 1996 and Rajaram *et al.*, 1997). Interestingly, PSA has been shown to be a protease that cleaves IGFBP-3, resulting in an increase of free IGF-1 in
25 serum (Cohen *et al.*, 1992; Cohen *et al.*, 1994; Lilja, 1995). Clearly, regulation of IGF-1R activity can play an important role in several disease states, indicating that there are potential clinical applications for both IGF-1 agonists and antagonists.

 The type-1 insulin-like growth-factor receptor (IGF-1R) and insulin
30 receptor (IR) are related members of the tyrosine-kinase receptor superfamily of growth factor receptors. Both types of receptors are

INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

I. FIELD OF THE INVENTION

This invention relates to the field of hormone receptor activation or inhibition. More specifically, this invention relates to the identification of
5 molecular structures, especially peptides, which are capable of acting at either the insulin or insulin-like growth factor receptors as agonists or antagonists. Also related to this invention is the field of molecular modeling whereby useful molecular structures are derived from known structures.

II. BACKGROUND OF THE INVENTION

10 Insulin is a potent metabolic and growth promoting hormone that acts on cells to stimulate glucose, protein, and lipid metabolism, as well as RNA and DNA synthesis. A well-known effect of insulin is the regulation of the level of glucose at a whole body level. This effect by insulin occurs predominantly in liver, fat, and muscle. In liver, insulin stimulates glucose
15 incorporation into glycogen and inhibits the production of glucose. In muscle and fat, insulin stimulates glucose uptake, storage, and metabolism. Disruptions of glucose utilization are very common in the population in giving rise to diabetes.

Signal transduction in target cells is initiated by binding of insulin to a
20 specific cell-surface receptor, the insulin receptor (IR). The binding leads to conformational changes in the extracellular domain of the receptor, which are transmitted across the cell membrane and result in activation of the receptor's tyrosine kinase activity. This, in turn, leads to autophosphorylation of the insulin receptor's tyrosine kinase, and the
25 binding of soluble effector molecules that contain SH2 domains such as phosphoinositol-3-kinase, Ras GTPase-activating protein, and phospholipase C γ to IR (Lee and Pilch, 1994).

Insulin-like growth factor 1 (IGF-1) is a small, single-chain protein (MW = 7,500 Da) that is involved in many aspects of tissue growth and

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(54) Title: INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

(57) Abstract: Peptide sequences capable of binding to insulin and/or insulin-like growth factor receptors with either agonist or antagonist activity and identified from various peptide libraries are disclosed. This invention also identifies at least two different binding sites which are present on insulin and insulin-like growth factor receptors which selectively bind the peptides of this invention. As agonists, certain of the peptides of this invention may be useful for development as therapeutics to supplement or replace endogenous peptide hormones. The antagonists may also be developed as therapeutics.

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